

The role of diet in clinical and endocrine manifestations of *acne vulgaris*

A thesis submitted in complete fulfilment of the requirements for
the degree of Doctor of Philosophy

Robyn N. Smith
BAppSci (Hons)

School of Applied Sciences
Science, Engineering and Technology Portfolio
RMIT University
Melbourne, Australia
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DECLARATION

I hereby declare that the thesis entitled “The role of diet in clinical and endocrine manifestations of *acne vulgaris*”, submitted for the Degree of Doctor of Philosophy, is the result of my own research, except where otherwise acknowledged, has been conducted since the official commencement date of the approved program; and has not been submitted previously, in whole or in part, to qualify for any other academic award.

Signature : _____
Robyn Nicole Smith

Date: _____ August 15th 2008

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From the dusty library archives she surfaces.....

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CANDIDATES ROLE IN STUDY

Study 1) Controlled feeding (pilot) study – conducted at Ballarat University (Chapter 2)

Study 2) Outpatient dietary intervention trial – conducted at RMIT University (Chapters 3-6)

| ACTIVITY | PERFORMED BY (Study number) |
|--|--|
| Study design and supervision | NM (1,2), AB (1,2), GV (1,2), RS (1,2) |
| Study co-ordination | RS (1,2) |
| Subject recruitment | RS (1,2) |
| Subject screening | RS (1,2) |
| Dermatology assessment training | AB (2), GV (2) |
| Dermatology assessments | AB (1,2) |
| Dietary counselling | RS (2) |
| Menu plan | RS (1) |
| Food provision and preparation | RS (1,2), HM (1), JR(1) |
| Venepuncture | RS (1,2), LM (2) |
| Blood glucose measurements | HM (1,2), NF (2), LW (2) |
| Anthropometric measurements | RS (1,2) |
| Dietary data entry | RS (1,2) |
| Glycemic load calculations | RS (1,2) HM (2) |
| Plasma/serum processing | AJ (1,2), YP (1,2), RS (1) |
| Lipid analysis | RMH (1,2) |
| Hormone analysis (insulin, sex hormones) | RMH (1,2) |
| Growth factor analysis | RMH (1,2) |
| IGFBP-1 & -3 analysis | RS (1,2), SS (1,2) |
| Plasma fatty acid analysis | RS (2) |
| Sebum fatty acid analysis | RS (2) |
| Sebutape application | HM (2), RS (2) |
| Sebutape photometric analysis | RS (2) |
| Statistical advice | DK (1,2), JG (1) |
| Statistical analysis | RS (1,2) |
| Manuscript writing and preparation | RS (1,2) |

RS Robyn Smith - School of Applied Sciences, Food Science, RMIT University
 NM Neil Mann – School of Applied Science, Food Science, RMIT University
 AB Anna Braue – Dept of Dermatology, Royal Melbourne Hospital
 HM Henna Mäkeläinen - Dept of Biochemistry and Food Chemistry, Turku University, Finland
 GV George Varigos – Dept of Dermatology, Royal Melbourne Hospital and Royal Children’s Hospital
 JR Jessica Roper – School of Applied Science, Food Science, RMIT University
 EM Elizabeth Moore – Dept of Dermatology, Royal Melbourne Hospital
 NF Nicole Fitzpatrick – School of Applied Science, Food Science, RMIT University
 LW Leah Williamson – School of Applied Science, Food Science, RMIT University
 AJ Ali Jafarpour – School of Applied Science, Food Science, RMIT University
 YP Yvonne Pirota – School of Applied Science, Food Science, RMIT University
 SS Sri Smeka – Kolling Institute of Medical Research, Royal North Shore Hospital
 DK Dan Kildea - School of Mathematics, Statistics, RMIT University
 JG John Glass - School of Applied Science, Food Science, RMIT University
 RMH Royal Melbourne Hospital – Pathology Dept

LIST OF ABBREVIATIONS

| | |
|----------|--|
| Δ | Delta |
| α | Alpha |
| β | Beta |
| Acne-QoL | Acne-specific quality of life |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| AR | Androgen receptor |
| BMI | Body mass index |
| CI | Confidence interval |
| cm | Centimetre |
| CV | Coefficient of variation |
| DHEA | Dehydroepiandrosterone |
| DHEA-S | Dehydroepiandrosterone sulphate |
| DHT | Dihydrotestosterone |
| FAME | Fatty acid methyl esters |
| FAI | Free Androgen Index |
| GI | Glycaemic index |
| GL | Glycemic load |
| GnRH | Gonadotropin releasing hormone |
| GH | Growth hormone |
| HDL | High density lipoprotein |
| HGL | High glycemic load |
| HOMA-IR | Homeostasis model of insulin resistance |
| HSD | Hydroxysteroid dehydrogenase |
| iAUC | Incremental area under the curve |
| IGFBP-1 | Insulin-like growth factor binding protein-1 |
| IGFBP-3 | Insulin-like growth factor binding protein-3 |
| IGF-I | Insulin-like growth factor-I |
| IFN | Interferon |
| IL | Interleukin |
| IR-KO | Insulin receptor-knock out |
| kJ | Kilojoules |
| LTB4 | Leukotriene B4 |
| LDL | Low density lipoprotein |
| LGL | Low glycemic load |
| mg | Milligram |
| MJ | Megajoule |
| mL | Millilitre |
| mRNA | Messenger ribonucleic acid |
| MUFA | Monounsaturated fatty acids |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NEFA | Non-esterified free fatty acids |
| ng | Nanogram |

| | |
|---------------------|--|
| nmol | Nanomoles |
| OGIS | Oral glucose insulin sensitivity index |
| OGTT | Oral glucose tolerance test |
| <i>P.acnes</i> | Propionibacterium acnes |
| PCOS | Polycystic ovary syndrome |
| PGE2 | Prostaglandin E2 |
| PPAR | Peroxisome proliferator activator receptor |
| PUFA | Polyunsaturated fatty acids |
| QoL | Quality of life |
| RAR | Retinoic A receptor |
| RXR | Retinoic X receptor |
| SFA | Saturated fatty acids |
| SHBG | Sex hormone binding globulin |
| SEM | Standard error of the mean |
| tAUC | Total area under the curve |
| T-Chol | Total cholesterol |
| TNF | Tumour necrosis factor |
| wk | Week |
| yr | Year |
| | |
| C14:0 | Myristic acid |
| C16:0 | Palmitic acid |
| C16:1 Δ 6 | Sapienic acid |
| C16:1 Δ 9 | Palmitoleic acid |
| C18:0 | Stearic acid |
| C18:2 Δ 9,12 | Linoleic acid |
| C20:0 | Arachidic acid |

PREFACE

To understand the role of food in acne, it's important to understand how the science of nutrition has changed. During much of the 20th century, the nutritional challenge was the prevention of dietary deficiencies. This led to the establishment of essential nutrients and dietary guidelines for the purpose of preventing deficiencies and of supporting normal growth and maintenance. During the late 20th and early 21st century, the societal abundance, which characterises most of the Western World, threw up new nutritional challenges. The concept of nutrition progressed from preventing dietary deficiencies to recommendations aimed at avoiding excessive consumption of some nutrients, after recognising their role in the aetiology of disease. Public awareness of the cause-and-effect relationship of the role of food in general health has caused an explosion in the scientific and mainstream literature, and the development of new nutritional concepts, such as *functional foods* to reduce the risk of disease and improve quality of life. This megatrend, actively popularised by mainstream media, and the dramatic changes in the attitudes of consumers, has fuelled an explosion in nutritional research.

Given the logarithmic progression in the science of nutrition, there is merit in reassessing the role that diet plays in acne development. Forty years ago, at the time of the last diet and acne study, many authorities found the concept of food causing disease, when no metabolic or nutritional deficiency exists, difficult to comprehend. This reflects the consideration at the time of minimum dietary requirements as a means of defining nutritional guidelines. Fulton *et al* (1969) stated that “if a food can really alter a disease..[this] finding would set into motion a wholesale attack on the effects of foods on normal physiologic functions.” This statement highlights a principal difference between the science of nutrition in 1969 and that of today. Since the advent of functional foods, modern-day nutritional research is largely directed towards understanding how the food we eat relates to health or particular pathologic processes. Nutrition has come to the fore of mainstream modern medicine and doctors are being asked now more than ever for unbiased information on the effects of certain diets, foods, supplements and numerous other dietary elements on health and disease.

While progress has been made over the past 40 years in the nutritional field, it is distressing to realise how little our understanding of the relationship of acne to foods has changed. There are surprisingly few studies that examine the role of diet in acne development. There are no meta-analyses, randomised controlled clinical studies, or well-designed scientific trials that follow evidence-based guidelines for providing solid proof in dealing with this issue. Despite the lack of hard evidence, the current consensus amongst most of the medical profession is that diet did not cause pimples. In the popular press, article after article lists “acne is caused by diet” as “Myth #1”. However, few doctors feel that they can argue fervently on the issue (Nash,

2003) and many are quite surprised at the poor quality of the studies on which this myth is based (Dr Anne Howard, personal communication).

The following thesis reconsiders the diet and acne connection, and opens a new chapter for an understanding of nutrition on skin health. According to Yudkin (1953), there are three criteria by which one tests the hypothesis that a dietary component is implicated in the causation of disease. “Firstly, there should be evidence that the diet of persons with the disease differs significantly from that of persons without the disease. Secondly, the symptoms and signs should be those that are known to be, or plausibly suspected of being, caused by dietary imbalance. Thirdly, correction of the dietary imbalance should result in correction of the signs and symptoms.” Although these fundamental principles should be simple to apply, the major limitation 50 years ago was a poor understanding of the cause and exacerbation of acne. During the past 30 years, significant advances have been made in the area of dermatology, subsequently acne has begun to be characterised at each stage of the disease process. A greater understanding of the disease process has enabled a more mechanistic and pathophysiological approach to understanding the role that diet plays in acne development.

Recent studies on the variation of acne prevalence worldwide have prompted researchers to question the natural development of the disease. In 1969, Fulton *et al* stated that “it would be remarkable if skin functions were easily influenced by the vagaries of the diverse diets which have evolved in human populations.” Today, however, nutritionists are increasingly convinced that dietary habits have significantly contributed to the proliferation of several chronic disease states, such as diabetes and cardiovascular disease, in Western countries. So, perhaps, what was once deemed remarkable in 1969 is now comprehensible in 2008. There is now also compelling evidence from epidemiological studies to suggest a potential role of diet in acne development. Ultimately, however, only experimental and clinical studies can confirm the biological consequences of dietary choices. This thesis provides for the first time clinical evidence of a cause-and-effect relationship between diet and acne and hopefully will provide greater insight into the potential underlying mechanisms involved.

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CHAPTER ONE

Acne and the Western diet

An examination of the potential mechanisms involved

Robyn Smith¹ and Neil Mann^{1,2}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Australian Technology Network, Centre for Metabolic Fitness

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1.1 ABSTRACT

In Western civilisations, acne is a common skin disease affecting the majority of the adolescent population. Despite its high prevalence in the West, acne incidence is considerably lower in non-Westernised societies and prevalence rates have been shown to increase when a previously unaffected society adopts a Western lifestyle. These observations urge further examination of the lifestyle factors that may influence the pathological mechanisms involved in acne. Among the possible lifestyle factors (eg. diet, physical activity, climate, stress, sun exposure), diet is a likely contender based on observational evidence. This paper explores the possible diet-acne connection by examining factors that differentiate diets of non-Westernised societies from those of the West.

Nutritional research has revealed that non-Westernised societies consume fewer high glycaemic index carbohydrates, dairy foods and have lower intakes of omega-6 polyunsaturated oils than Westernised societies. These dietary factors may affect the development of acne through their influence on mediators such as androgens, sex-hormone binding globulin, insulin-like growth factor (IGF)-I, IGF-binding proteins and inflammatory leukotrienes and prostaglandins. Together, these may affect several aspects of the disease process including sebum production, desquamation of keratinocytes and the surrounding inflammatory response. Consequently, this review proposes that Western diets may play a multifactorial role in the pathogenesis of acne.

1.2 INTRODUCTION

1.2.1 *Acne vulgaris: Definition and pathophysiology*

Acne vulgaris is a complex disorder of the pilosebaceous unit, which is comprised of sebum-producing sebaceous glands, a keratinocyte-lined follicular canal and a hair follicle. Acne is thought to arise from the interplay of four pathological factors: (i) increased growth and abnormal differentiation of follicular keratinocytes which obstruct the follicle opening; (ii) increased sebum production in response to androgen hormone stimulation; (iii) increased bacterial colonisation by *Propionibacterium acnes* (*P.acnes*), and (iv) the host immune response due to the increased numbers of bacteria and rupturing of the follicular wall (Brown and Shalita, 1998; Thiboutot, 2000; Gollnick, 2001). These events can lead to the formation of non-inflammatory lesions and /or inflammatory papules, pustules and nodules.

1.2.2 *Acne prevalence and factors affecting acne incidence rates*

In Western civilisations, acne is widespread with prevalence rates ranging between 70-87% for adolescents (Kilkenny *et al.*, 1997; Dreno and Poli, 2003). Following puberty, acne prevalence decreases with age, with only 8% of adults aged 25-34yr and 3% of those aged 35-44yr affected (Bergfeld, 1996). Acne prevalence also varies between the sexes, as the disease is more common and severe in males (Stathakis *et al.*, 1997). However, women are more likely to have acne persist into adulthood, with reports indicating as many as 12% of women over 25yr are affected (Goulden *et al.*, 1997; Goulden *et al.*, 1999b).

Other factors can influence the incidence of acne in various subpopulations. Variations in acne prevalence exist according to genetics (family, ethnicity, gender), socioeconomic status and life settings such as urban vs. rural (Lehmann *et al.*, 2001). In a multi-ethnic study in the USA, acne prevalence was highest in Hispanics (79.2%), while similar rates were reported amongst African Americans (59.9%) and Asian subpopulations (63.2%) (Taylor *et al.*, 2002). These findings, together with observations of the concordance of acne in monozygotic twins, further highlight that acne may have a genetic component (Walton *et al.*, 1988; Goulden *et al.*, 1999a). However, acne prevalence in Kenya (Ormsby and Montgomery, 1954), Zambia (Ratnam and Jayaraju, 1979) and Peru (Freyre *et*

al., 1998) is reportedly lower than in the black (Wilkins and Voorhees, 1970; Taylor *et al.*, 2002) and Hispanic (Taylor *et al.*, 2002) populations of Western countries, suggesting that environmental factors play a role. Furthermore, a recent Turkish study suggested that socioeconomic status may be a factor, after acne was more frequently observed in children of affluent communities (Inanir *et al.*, 2002). Given the evidence from these studies, it is plausible to suspect that acne may develop in genetically predisposed individuals in response to environmental triggers. The environmental factors responsible for the onset of acne are unclear, however the impact of diet should be further explored.

1.3 DIET AND ACNE – EXPLORING THE LINK

During the last century, much controversy has surrounded the subject of diet in acne management. In the early 1950s, major dermatology textbooks recommended dietary modifications in the treatment of acne (Sulzberger and Baer, 1948; Belsario, 1951). Most advised against the excessive consumption of fat and carbohydrates, targeting foods such as chocolates, sweets, and carbonated beverages (Sulzberger and Baer, 1948; Belsario, 1951). However, apart from anecdotal accounts and case reports, little evidence could support the removal of such items from the diet (Cornbleet and Gigli, 1961; Rasmussen, 1977). Twenty years later, dietary advice was removed from standard texts and the consensus has since been that diet plays no role in the development of acne.

The most frequently cited reference dissociating diet and acne was the study by Fulton *et al.* (1969), which has since been widely criticised for its design and mode of evaluating acne (Mackie and Mackie, 1974; Rasmussen, 1977; Cordain, 2003; Treloar, 2003). This study compared the effects of a chocolate versus a placebo bar in a crossover double-blind study (Fulton *et al.*, 1969). After examining the data, the authors concluded that “ingestion of high amounts of chocolate did not materially affect the course of acne vulgaris” (Fulton *et al.*, 1969). However, a review of this work, found no real difference in the lipid and sugar contents of the two bars (Mackie and Mackie, 1974). Furthermore, the fatty acid composition of the hydrogenated fat in the placebo was very similar to that of cocoa butter (Mackie and Mackie, 1974). The authors described the chocolate study as “incorrectly controlled”, stating that the results were “not valid”.

Two years later, a study involving US college students set out to examine whether certain foods aggravate or worsen acne. Twenty-seven students consumed the culprit foods everyday for a week and returned daily to an outpatient clinic to be examined. No major flares of acne were observed. However, if aetiological mechanisms are to be suspected, it is unlikely that they would produce a clinical response given the study's short-duration. Recently, Treloar (2003) also questioned the validity of the study design, the small sample size and the absence of baseline dietary information.

There is no compelling scientific evidence to indicate that individual foods cause acne, and the established medical view is that "diet plays no role in acne treatment in most patients" (Kaminester, 1978). However, this advice is based upon these poorly controlled studies which focus on individual foods and fail to consider the influence of other dietary factors. No food has been identified as single cause of other Western diseases, such as diabetes, atherosclerosis and cardiovascular diseases, which are now accepted as nutritionally-related disorders. In fact, for many years opponents disregarded diet as a causative factor in heart disease, as dietary histories failed to predict serum cholesterol levels or coronary heart disease mortality (Kannel and Gordon, 1970; Nichols *et al.*, 1976; Rosenberg and Kirk, 1981). However, characterising the role of various dietary factors (eg. saturated fat, glycaemic index and fibre) and their physiological effects has since increased our understanding of their impact on cardiovascular disease health (Reaven, 1995; Kris-Etherton *et al.*, 2002; Ludwig, 2002; Cordain *et al.*, 2005). When one compares the wealth of literature on the 'diet-heart' theory with that of the acne, it is clear the nutritional research into acne is still very much in its infancy.

1.3.1 *Nutrition and diseases of the West*

In most Western countries, diet-related chronic diseases represent the single largest cause of morbidity and mortality. As diseases such as type 2 diabetes, cardiovascular disease and obesity reach epidemic proportions in Western nations, they remain rare in non-industrialised or less Westernised communities (Eaton *et al.*, 1988; Lindeberg *et al.*, 2003b; O'Keefe and Cordain, 2004). Extensive pathophysiologic research has identified many environmental and dietary factors that are influential in disease aetiology, including caloric intake, tobacco use, physical inactivity, glycaemic load, fibre intake, fatty acid composition, macronutrient composition and salt intake (Eaton *et al.*, 1988; Cordain

et al., 2005). Altogether, these diet and lifestyle factors along with genetic susceptibility may provide a complex interaction in the pathophysiology of Western disease. Therefore, from our current understanding of nutrition it is unlikely that acne would arise from the excessive consumption of single foods, but rather a complex interaction of many dietary/lifestyle factors as do other non-communicable diseases.

It has recently been proposed that diets consumed by non-Westernised societies may serve as models for protection against acne. Researchers have previously explored the diet-acne connection by reporting the emergence of acne in Eskimos of Northern America following the adoption of a Western lifestyle (Schaefer, 1971; Bendiner, 1974). Acne was virtually absent among the Eskimo youth as they continued to maintain their hunter-gatherer lifestyle (Schaefer, 1971; Bendiner, 1974). The diet of Eskimos in earlier times was based almost entirely on animal protein and fat, with land and marine animals constituting 80-100% of their food intake (Nestle, 1999). However, when they urbanised and were exposed to a high carbohydrate diet, acne became a common and sometimes severe problem. Although the primary determinant for the increased acne incidence is unknown, the Eskimos reportedly blamed the increased sugar consumption for the appearance of acne (Schaefer, 1971). In addition to acne, other common Western diseases also emerged, including dental caries, obesity, diabetes and heart disease (Schaefer, 1971; Bendiner, 1974).

Cordain *et al* (2001) recently proposed that freedom from acne was related to the differences in the digestibility of carbohydrate in the diet. This concept is supported in their observations of the Ache' hunter-gatherers of Paraguay and the Kitavan subsistence horticulturists of Papua New Guinea. In over 1300 subjects (aged 15-25yr), not a single case of acne was reported. The Kitavan and Ache societies ate foods such as low glycaemic index (GI) tubers, fruits, fish and coconut and locally cultivated foods (sweet manioc, peanuts, maize and rice) (Cordain *et al.*, 2002). They were also more physically active than their Western counterparts and did not show any signs or symptoms of other chronic Western diseases, such as diabetes and/or cardiovascular disease (Lindeberg *et al.*, 1999). The authors attribute the absence of acne to environmental factors, mainly local diets, which are devoid of high GI carbohydrates. They also argued that genetic factors are not wholly responsible for the observed

differences, as other South American Indians and Pacific Islanders, who share similar ethnic backgrounds, have higher levels of acne when consuming a Western diet (Cordain *et al.*, 2002).

Epidemiologists have defined the primary cause of disease, as not one in whose presence disease will always occur, but as one in whose absence disease will not occur (Rosenberg and Kirk, 1981). In line with this concept, Cordain *et al* (2001) attributed the absence of acne to a diet devoid of high GI carbohydrates. However, there are many factors that dissociate traditional human diets from those of the Western civilisations. Consequently, this review attempts to identify dietary elements that are pervasive in Western societies, but remain rare or unfamiliar to traditional societies.

1.3.2 Glycaemic index and glycaemic load

Cordain *et al* (2001) observed that the carbohydrates eaten by the Ache and Kitavan societies were uniformly low in GI, which is a relative scale used to determine the blood glucose-raising potential for a given sample of food containing 50g of available carbohydrate (Jenkins *et al.*, 2002). By definition, high GI carbohydrates are rapidly digested, producing rapid, high concentrations of blood glucose and increasing insulin demand. In contrast, low GI carbohydrates are slowly digested and absorbed during the postprandial period. It is thought that the use of low GI foods in traditional cultures may play a protective role against Western disease. This notion is supported by the observed high rates of type 2 diabetes in the Pima Indians and Australian aborigines for whom the dietary transition to high GI foods has been a fairly recent phenomenon (Thorburn *et al.*, 1987; Boyce and Swinburn, 1993; Colagiuri and Brand-Miller, 2002).

A unifying feature of traditional diets is that they are naturally low in glycaemic load (GL). As the GI can only be used to compare foods of equal carbohydrate content, the GL was developed to characterise the glycaemic effect of meals or diets of differing macronutrient composition. The GL takes both the quantity and quality of carbohydrate into consideration and is calculated as $GI \times \text{carbohydrate content per serve}$. The Eskimo's diet, which was traditionally high in protein and fat with minimal carbohydrate, would have been low in GL due to the limited availability of carbohydrate

(Schaefer, 1971). Conversely, the Ache and Kitavan diets were high in carbohydrate (>60% total energy), but low in GL due to exclusive consumption of low GI foods.

There is suggestive evidence that the GL of Western diets has increased over recent years. Studies of dietary trends between 1989-1996 and 1971-2000 in the US indicate that carbohydrate intake has increased (Cavadini *et al.*, 2000; Wright *et al.*, 2004). The increase in carbohydrate consumption is thought to be a consequence of dietary recommendations to decrease dietary fat (Slyper, 2004). Several studies have demonstrated that a reduction in dietary fat is invariably associated with increased consumption of simple sugars (Gibney, 1990; Nicklas *et al.*, 1992). Slyper (2004) also suggested that the overall dietary GI has increased in children's diet over the last few decades. There have been significant increases in the consumption of high GI foods, such as ready-to-eat cereals (Nicklas *et al.*, 1998), potatoes (Cavadini *et al.*, 2000), soft drinks (Troiano *et al.*, 2000), and salty snacks (Nielsen *et al.*, 2002), with considerably more foods being consumed outside the home (Nielsen *et al.*, 2002). A high carbohydrate diet, based on rapidly digestible carbohydrates, would repeatedly expose individuals to acute elevations in blood glucose and insulin. Cordain *et al* (2001) proposed that diet-induced hyperinsulinaemia may elicit an endocrine response that simultaneously promotes follicular epithelial growth and enhanced sebaceous gland activity – two factors responsible for acne proliferation. Therefore, it is possible that a high dietary GL may account for higher prevalence of acne in Western societies.

1.3.3 *Essential fatty acids*

There is evidence to suggest that essential polyunsaturated fatty acids (PUFA) also play a role in acne pathogenesis (Logon, 2003). Of particular importance is the dietary balance of two series of PUFA: the omega-6 (which are generally proinflammatory) and the omega-3 fatty acids (which are anti-inflammatory with several intrinsic cardioprotective effects). In Western diets, the ratio of omega-6/omega-3 fatty acids is generally high (of the order 20:1), due to a high intake of vegetable oils and consumption of grain-fed livestock (Cordain *et al.*, 2005). High intakes of omega-6 is known to increase levels of lipid inflammatory mediators, such as leukotrienes and prostaglandins, and this can have adverse effects on skin disorders (Horrobin, 2000; James *et al.*, 2000). Lindeberg *et al* (2003a)

previously described the lower intake ratio of omega-6/omega-3 PUFA in the Kitavan population compared with Western populations and a low intake ratio (approximately 2:1) is also a characteristic feature of hunter-gatherer societies such as the Ache' population (Simopoulos, 1999). Consequently, the lower dietary ratio of omega-6/omega-3 PUFA may also help to explain the absence of acne in traditional societies.

1.3.4 Dairy intake

Another distinguishing feature of traditional diets is the absence of dairy foods (Cordain, 2003). A recent retrospective evaluation of dairy intake revealed a positive association between milk intake (total and skim) and physician-diagnosed severe acne (Adebamowo *et al.*, 2005). The authors suggest that hormonal constituents of milk (estrogens, androgen precursors and IGF-I) are present in sufficient quantities to exert biological effects on the hormone-sensitive pilosebaceous units. However, whether IGF-I can retain its bioactivity and be protected from proteolysis in the gut is still uncertain. The intestinal absorption of milk-borne IGF-I is negligible in animals, however no such trials have been demonstrated in humans (Burrin, 1997; Donovan *et al.*, 1997). Furthermore, androgen intake from milk is very low (0.06% of circulating testosterone is from milk) and these estimated intakes could be further reduced by androgen metabolism in the liver (Adebamowo *et al.*, 2005). However, another possible explanation is the highly insulintropic nature of dairy products (Holt *et al.*, 1997; Liljeberg Elmståhl and Björck, 2001; Östman *et al.*, 2001). Dairy products have a 3- to 6- fold greater insulin response compared with foods with similar GI values (Östman *et al.*, 2001). Therefore, it is possible that the absence of dairy foods in traditional societies may also play a role in the negligible acne seen in these societies.

1.4 INSULIN RESISTANCE AND HYPERINSULINAEMIA

An important factor differentiating diets of more traditional societies from that of the West is that several Western dietary components (GI, carbohydrate intake, dairy foods) have the potential to lead to postprandial hyperinsulinaemia. However, hyperinsulinaemia can also be a function of the body's reduced sensitivity to plasma insulin. When peripheral tissues become resistant to the insulin-mediated uptake of glucose, clinically defined insulin resistance occurs. Hyperinsulinaemia is commonly

coupled with insulin resistance, as more insulin is required to overcome tissue insensitivity so one can remain in a euglycaemic state (Livingstone and Collison, 2002). The development of impaired glucose tolerance or type 2 diabetes marks a failure of the pancreas to maintain euglycaemia and blood glucose begins to rise in a pathological fashion (Cordain *et al.*, 2003). Several factors are known to influence insulin resistance including age, physical fitness, obesity, genetics and foetal or infant malnutrition (Liese *et al.*, 1998; Reaven, 1998).

Colagiuri and Brand Miller (2002) hypothesised that insulin resistance may have provided survival and reproductive advantages to our ancestors who were genetically adapted to a carnivorous-type diet. In their natural environment, it is thought that insulin resistance would facilitate the deposition of food energy as body fat in preparation for times of food scarcity (“thrifty genotype”) (Neel, 1962). Insulin resistance would not have resulted in compensatory hyperinsulinaemia with the consumption of a high protein, low carbohydrate diet (Colagiuri and Brand-Miller, 2002). Although the introduction of agriculture over the last 10,000 years has caused a progressive and rapid dietary replacement of protein with carbohydrate, these carbohydrates were mostly low in GI (Colagiuri and Brand-Miller, 2002). The advent of milling processes to refine cereals with the industrial revolution made starch more digestible, increasing the postprandial glucose and insulin responses 2-3 fold compared to coarse or unrefined grains (Brand *et al.*, 1985; Heaton *et al.*, 1988; Colagiuri and Brand-Miller, 2002). The consequence of this last dietary transition was that insulin resistance became associated with significant hyperinsulinaemia, a combination which plays a role in several chronic disease states (Reaven, 1998).

High GL load diets have been implicated in the deterioration of insulin sensitivity and the decline in β -cell function which are critical to the development of type 2 diabetes (Brand-Miller, 2004). Several epidemiological and prospective studies have demonstrated an association of type 2 diabetes with high GL diets (Salmeron *et al.*, 1997a; Salmeron *et al.*, 1997b; Schulze *et al.*, 2004). However, examining the effect of diet on insulin resistance is difficult because many other factors influence insulin action and it often goes undiagnosed until it reaches the advanced diabetic stage. An important principle in endocrinology is that of receptor down-regulation. A diet which persistently elevates insulin

concentrations may be expected to downregulate insulin receptors, thereby resulting in insulin resistance (Virkamaki *et al.*, 1999). Feeding rats a high GL diet (high in glucose or amylopectin) has been shown to induce rapid and severe insulin resistance when compared to a low GL diet (high in amylose) (Byrnes *et al.*, 1994; Higgins *et al.*, 1996). Furthermore, evidence suggests that high GI foods may also affect insulin resistance through pathophysiologic mechanisms related to increases in free fatty acids (Boden *et al.*, 1991; Piatti *et al.*, 1991) and other obesity-related factors (eg. hyperphagia and fuel partitioning) (Brand-Miller *et al.*, 2002).

1.4.1 *Metabolic syndrome*

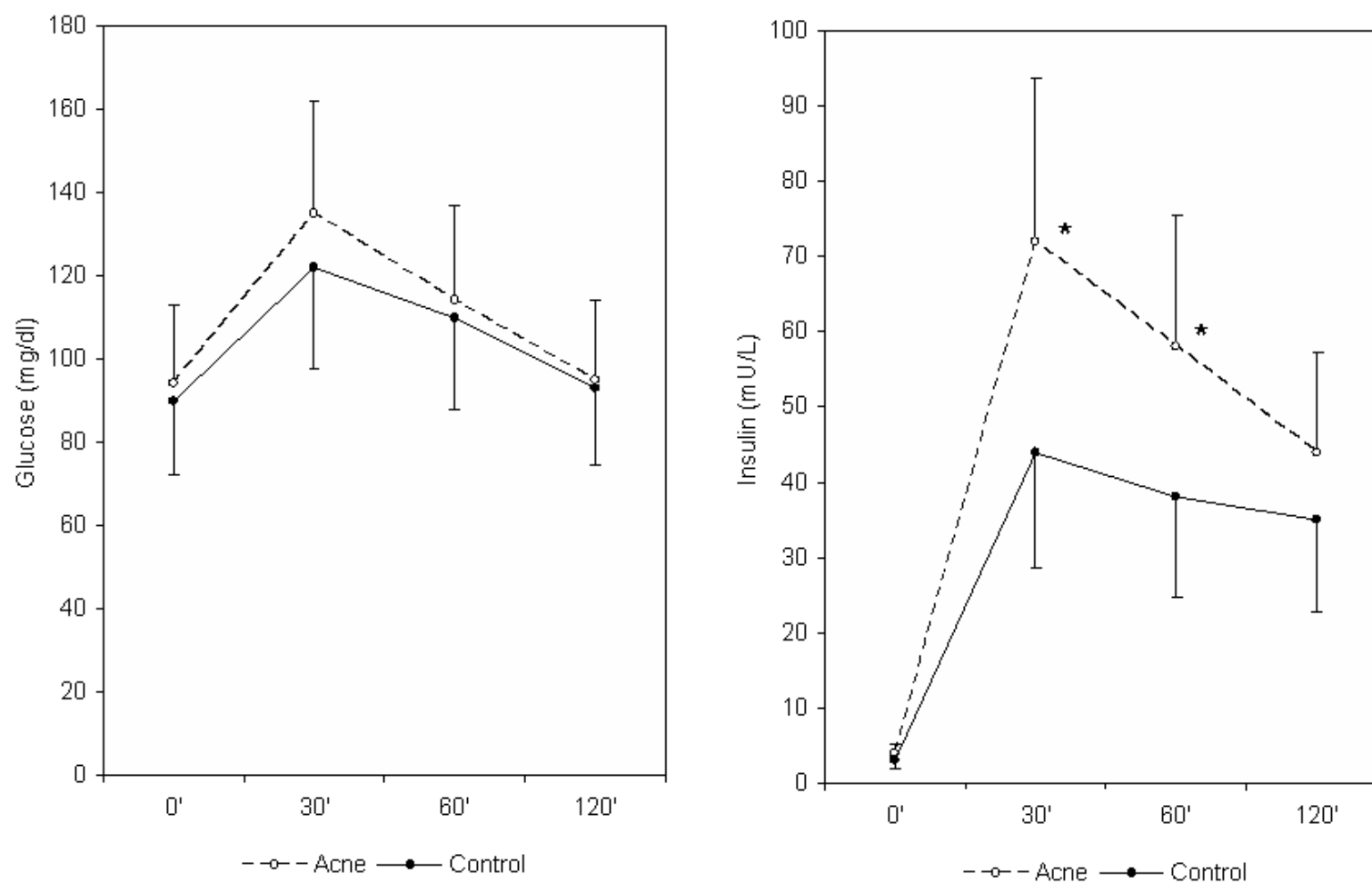
Insulin resistance and its compensatory hyperinsulinaemia are central to a number of metabolic abnormalities which are collectively termed ‘metabolic syndrome’ (Reaven, 1998). This syndrome is characterised by conditions of disturbed glucose and insulin metabolism, obesity, intra-abdominal fat, mild dyslipidaemia, hypertension and the subsequent development of type 2 diabetes and/or cardiovascular disease. However, the clinical consequences extend far beyond the realm of risk factors associated with type 2 diabetes and cardiovascular disease. Other maladies include cutaneous papillomas (skin tags), acanthosis nigricans, myopia, polycystic ovarian syndrome and certain cancers (breast, colon and prostate) (Cordain *et al.*, 2003). Cordain *et al.* (2003a) recently proposed that insulin resistance may also play an underlying role in acne pathogenesis.

1.4.2 *Evidence of the association between insulin resistance and acne vulgaris*

The link between acne and insulin resistance is not entirely unfamiliar, as acne is a clinical feature of polycystic ovarian syndrome (PCOS), a condition which affects women and is associated with insulin resistance, hyperinsulinaemia and hyperandrogenism (Falsetti *et al.*, 2002). Treatments for PCOS now includes insulin-sensitising drugs, which improve insulin sensitivity, restore fertility and alleviate acne (Kolodziejczyk *et al.*, 2000; Harborne *et al.*, 2003). Pharmacologic approaches to slow down carbohydrate absorption, notably with the use of α -glucosidase inhibitors, have also demonstrated significant improvements in acne in PCOS (Ciotta *et al.*, 2001). This tends to suggest that reducing the rate of carbohydrate absorption *per se* may help to ameliorate acne. However, whether the

consumption of natural unrefined, slow-release carbohydrates can achieve the same outcome remains unknown.

Disturbances in glucose metabolism were traditionally thought to be associated with acne. Numerous studies explored the relationship between fasting hyperglycemia and acne (Levin and Kahn, 1922; McGlasson, 1923; Strickler and Adams, 1932), however support for this relationship was inconsistent. Likewise, the results from glucose tolerance tests tended to be variable, often with unexplained variations from normal (Rost, 1932; Cornbleet and Gigli, 1961) to borderline (Greenbaum, 1930) to reduced tolerance (Semon and Herrmann, 1961). However, these studies looked at glucose concentrations at specific time points without any reference to insulin. This could have been a significant limitation as glucose tolerance is, in part, determined by sensitivity of peripheral tissues to insulin. This is supported by a more recent study which found the glucose response profile to be similar in acne patients and non-acne sufferers, however the total insulin response (**Figure 1**) was significantly greater in acne patients (Aizawa and Niimura, 1996). The authors observed that the relationship between acne and hyperinsulinaemia was independent of obesity and menstrual irregularities.



* $P < 0.05$

Figure 1.1. Mean insulin and glucose concentrations during a 75g OGTT in women with postadolescent acne versus controls without acne. Controls were matched for BMI and age and acne sufferers were otherwise healthy with no menstrual irregularities (Adapted from Aizawa and Niimura, 1996).

1.5 CHANGES IN THE HORMONAL MILIEU OF PUBERTY

Acne is a complex disease due to the interplay of hormones that control the pilosebaceous unit, with the pituitary as the driving factor due to its influence on androgen production and hence sebum production. However, it is unlikely that androgens are operating alone. For although the androgen dependency of sebaceous glands is unequivocal, most acne patients have normal concentrations of plasma androgens (Lucky *et al.*, 1997). Furthermore, it is not known why acne typically subsides in the late teenage years, when androgen levels remain stable throughout adulthood (Rosenfield *et al.*, 2000). The evolution of acne more closely resembles the transient changes in insulin-stimulated glucose metabolism during puberty.

1.5.1 *Insulin resistance of puberty*

This pattern of pubertal development is normally associated with a transient increase in insulin resistance, with insulin stimulated glucose uptake decreased compared with adults and prepubertal children (Bloch *et al.*, 1987; Moran *et al.*, 1999). Previous studies using the euglycaemic insulin clamp have shown that the pattern of increasing insulin resistance begins during early puberty and increases across all Tanner stages (Amiel *et al.*, 1986; Moran *et al.*, 1999). According to cross-sectional observations, acne begins about the same time as the gradual increase in plasma insulin, the preadolescent rise in body mass index (BMI), and the increase in IGF-I serum levels (Caprio *et al.*, 1989; Smith *et al.*, 1989). Although causality has not been proven, there is indirect evidence to suggest that these metabolic changes during puberty may exaggerate factors involved in acne development.

The mechanism behind the metabolic deterioration in glucose control is unclear. Some studies suggest that the observed increase in body fat during puberty may contribute to the decrease in insulin sensitivity (Bloch *et al.*, 1987; Hoffman *et al.*, 2000). However, body fat increases occur prior to and post puberty, whereas the fall in insulin sensitivity is transient (Goran and Gower, 2001). Furthermore, other studies have shown that the relative fall in insulin sensitivity is not associated with changes in body fat (Moran *et al.*, 1999). Alternatively, pubertal insulin resistance may be driven by transient changes in growth hormone levels during puberty. Growth hormone (GH) pulse amplitude is highest

during mid-late puberty, reflecting an inverse relationship with pubertal changes in insulin sensitivity (Amiel *et al.*, 1986; Moran *et al.*, 2002). This increase in GH may contribute to insulin resistance via GH's effect on increasing lipolysis and free fatty acid concentrations (Goran and Gower, 2001). This is further supported by the fact that GH-deficient children have higher insulin sensitivities (Merimee *et al.*, 1967) and as GH treatment in GH-deficient adults results in the development of peripheral insulin resistance (Bramnert *et al.*, 2003).

1.5.2 Pubertal insulin resistance facilitates the effects of IGF-I

The observed pubertal change in insulin sensitivity may facilitate the growth promoting effects of the insulin-like growth factor (IGF) system. IGF-I plays an important role in stimulating cell proliferation and differentiation in a variety of tissues, as well as inhibiting cell apoptosis (Kaaks *et al.*, 2000). Serum IGF-I levels increase with advancing puberty and are associated with linear growth during this period (Rosen, 1999; Juul, 2003). GH, insulin and nutritional status (eg. caloric deprivation or excess) can all influence IGF-I production. GH stimulates the hepatic secretion of IGF-I and insulin enhances production by up-regulating GH receptor numbers (Lewitt, 1994; Thissen *et al.*, 1994). Nutritional status is also a critical regulator of IGF-I, as caloric restriction substantially lowers IGF-I levels (Thissen *et al.*, 1994; Giovannucci *et al.*, 2003). Although GH levels are increased during fasting, IGF-I secretion is reduced due to hepatic GH resistance (Merimee *et al.*, 1982). Caloric excess may increase IGF-I, but the effect of over-consumption is not nearly as strong as caloric restriction (Thissen *et al.*, 1994).

Insulin is also able to modulate the physiological effects of IGF-I through its influence on IGF-binding proteins (IGFBPs). The actions of IGFBPs can oppose those of IGF-I by binding to IGF-I, thus reducing its biological activity. IGFBP-1 plays an integral role in controlling free IGF-I and studies have shown that IGFBP-1 is a potent inhibitor of IGF-I effects *in vivo* (Clemmons, 1997). IGFBP-1 concentrations decrease during puberty and follow a close negative association with the rise in insulin resistance (Ibáñez *et al.*, 1997; Moran *et al.*, 2002). Insulin is the principle determinant of plasma IGFBP-1 levels and postprandial insulin can dramatically inhibit hepatic production of IGFBP-1 (Powell *et al.*, 1991). Conditions associated with elevated IGFBP-1 levels include the fasting state

(Cotterill *et al.*, 1993), exercise (Suikkari *et al.*, 1989) and poorly controlled insulin-dependent diabetes mellitus (Batch *et al.*, 1991). Travers *et al.* (1998) found an inverse relationship between IGFBP-1 levels and BMI in early pubertal children and suggested that this is most likely due to the positive association between BMI and insulin resistance (Travers *et al.*, 1998).

1.5.3 Insulin and IGF-I modulate androgenic activity

Insulin and IGF-I have been implicated in reproductive development through the activation of the hypothalamic-pituitary-gonadal axis (Burcelin *et al.*, 2003; Daftary and Gore, 2003). Homeostatic mechanisms related to sex steroid concentrations control the hypothalamic-pituitary-gonadal system through negative feedback. However, in cases of hyperinsulinaemia (eg. PCOS), there is exaggerated secretion of the gonadotropin, luteinizing hormone, suggesting an underlying hypothalamic defect and abnormal feedback sensitivity (Dunaif, 1997). Insulin resistance and hyperinsulinaemia may play a role in the genesis of this syndrome either through the direct effects of insulin or through insulin's ability to promote free IGF-I (Franks, 2002). IGF-I can increase gonadotropin releasing hormone (GnRH) mRNA expression (Zhen *et al.*, 1997) and the maturation of GnRH-expressing neuronal cells of the hypothalamus (Olson *et al.*, 1995), which may affect luteinizing hormone secretion from the pituitary. Insulin can also stimulate the secretion and expression of GnRH by hypothalamic neurons in culture (Burcelin *et al.*, 2003). Burcelin *et al.* (2003) noted that peripheral administration of insulin and the resultant hyperinsulinaemic state stimulated reproductive activity in intact peripubertal animals, (Burcelin *et al.*, 2003). Furthermore, improvements in insulin sensitivity with weight loss in overweight infertile women can decrease luteinizing hormone secretion and serum androgen concentrations (Burcelin *et al.*, 2003).

Insulin and IGF-I can also play a role in the tempo of the pubertal development through augmenting the bioavailability of sex steroids. During puberty, androgen levels rise, with testosterone being the biologically important extracellular androgen. Testosterone bioavailability is dependent on the serum concentration of the high-affinity binding protein known as sex-hormone binding globulin (SHBG) (Franks, 2003). Only free testosterone is biologically available to enter target tissues. Traditionally,

sex steroids were thought to be the major regulators of SHBG, but recent data suggest that nutritional factors, mediated by insulin, are more important in determining SHBG levels (Anderson, 1974; Franks, 2003). During puberty, hepatic production of SHBG declines simultaneously with the fall in insulin sensitivity (Holly *et al.*, 1989). Girls who experience early menarche have significantly lower SHBG levels from 10-15.9yr compared to girls with later menarche (Holly *et al.*, 1989). During puberty, serum SHBG concentrations are negatively associated with height and the degree of obesity (Holly *et al.*, 1989). IGF-I also inhibits SHBG secretion (Singh *et al.*, 1990; Crave *et al.*, 1995), which coincides with the inverse association of total IGF-I and SHBG during puberty. However, SHBG levels are more closely associated with IGFBP-I, suggesting that a common mechanism regulates both binding proteins. Low levels of SHBG are associated with conditions of insulin resistance including obesity (Crave *et al.*, 1995) and PCOS (Vermeulen *et al.*, 1996) and similar observations have been made for IGFBP-1 (Thierry Van Dessel *et al.*, 1999).

Insulin resistance is also an underlying feature of premature adrenarche, a condition which is predictive of later onset of acne (Miller *et al.*, 1996). Adrenarche refers to the pre-pubertal rise in the secretion of the adrenal steroids, DHEA, DHEA-S and androstenedione (Saenger and Dimartino-Nardi, 2001). Premature adrenarche is associated with higher levels of adrenal androgens than expected for age and is clinically diagnosed as having pubic hair at <8yr (Ibáñez *et al.*, 2000). Insulin resistance and hyperinsulinaemia are commonly associated with premature adrenarche (Oppenheimer *et al.*, 1995; Vuguin *et al.*, 2001; Denburg *et al.*, 2002), which suggests that insulin has an important role in normal adrenarche. Insulin and IGF-I receptors have been identified in the adrenal cortex and both can stimulate adrenal androgen production in human adrenocortical cells *in vitro* (Endoh *et al.*, 1996; Fottner *et al.*, 1998). Therefore, it is possible that insulin and IGF-I may act as nutritional signals in the modulation of adrenal androgen production *in vivo*.

As dietary trends of adolescents continue to favour high glycaemic foods, the fall in insulin sensitivity during puberty could predispose individuals to significant hyperinsulinaemia. This, in turn, can affect a multitude of hormonal changes (**Figure 1.2**) that occur prior to and during puberty: (i) sex steroid production, by amplifying GnRH; (ii) SHBG production by the liver; (iii) adrenal androgen

production; (iv) circulating IGF-I levels; and (iv) IGFBP-1 production. These shifts in endocrine systems may provide the hormonal stimuli for events leading to the development of acne.

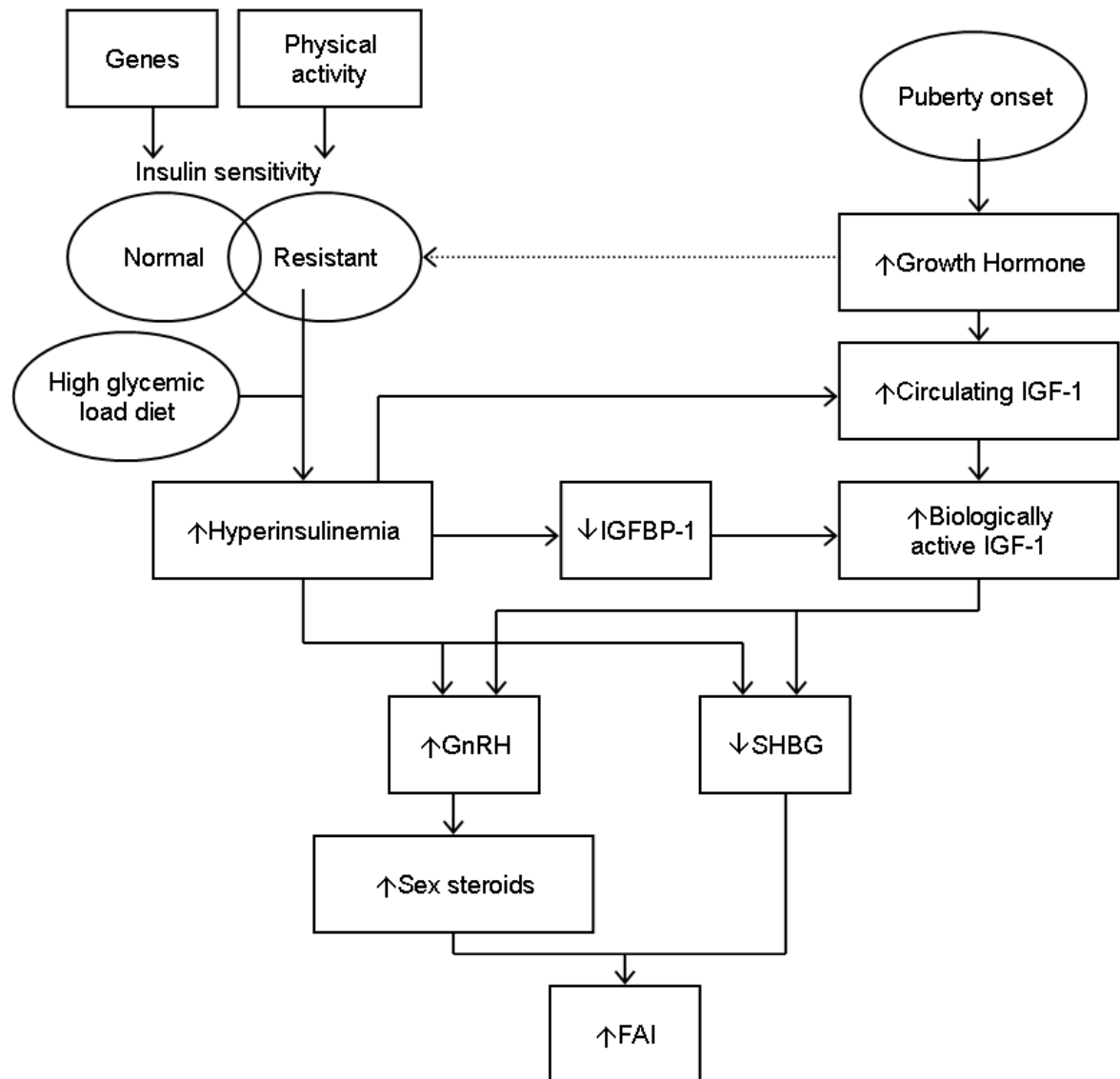


Figure 1.2: The combined effect of a high GL diet and pubertal insulin resistance on endocrine systems. As dietary trends of adolescents continue to favour high glycaemic foods, the fall in insulin sensitivity during puberty may be accompanied by significant hyperinsulinaemia. This may be facilitated by GH's effect on insulin sensitivity during puberty. The subsequent increase in plasma insulin levels may in turn encourage the growth promoting effects of the IGF-I by decreasing levels of IGFBP-1. Moreover, insulin may stimulate androgenic effects of sex hormones by decreasing SHBG levels and increasing sex steroid production through an increase in GnRH.

1.6 EXPLORING THE LINK BETWEEN NUTRITIONAL FACTORS AND ACNE PATHOGENESIS

Over the past thirty years, clinical research has characterised many of the physiological processes and immunological reactions involved at each stage of acne development. A better understanding of the disease at the biochemical level has helped to elucidate a possible role of nutritionally-related factors in acne aetiology. For example, there is now extensive clinical and experimental evidence to suggest that sex steroids and insulin interact in their actions on tissues. As discussed previously, insulin can also affect the changing hormonal milieu of puberty. Therefore, insulin may play a direct or indirect (via endocrine systems) role in the underlying molecular and non-inflammatory mechanisms involved in acne development. Furthermore, other nutritional factors (eg. proinflammatory fatty acids) may also influence the secondary inflammatory mechanisms that lead to the presentation of inflammatory acne. The following section attempts to illustrate how these nutritionally-related factors could play a role at each stage of the disease process.

1.7 SEBACEOUS GLAND DEVELOPMENT AND SEBUM PRODUCTION

The initial phase in the pathogenesis of acne is the androgen-induced enlargement of the sebaceous glands and increased production of sebum (Toyoda and Morohashi, 2001). Androgens are the major sebotrophic hormones and sebaceous glands are very sensitive to androgens whereas estrogens exhibit an inhibitory effect (Toyoda and Morohashi, 2001). Hormonal control of sebaceous gland function is complex and various factors influence sebaceous gland function such as androgen production, androgen bioavailability, peripheral androgen utilisation, or androgen receptor sensitivity (**Figure 1.3**). Furthermore, sebocyte proliferation and differentiation is also influenced by GH, IGF-I, and insulin (Shaw, 2002).

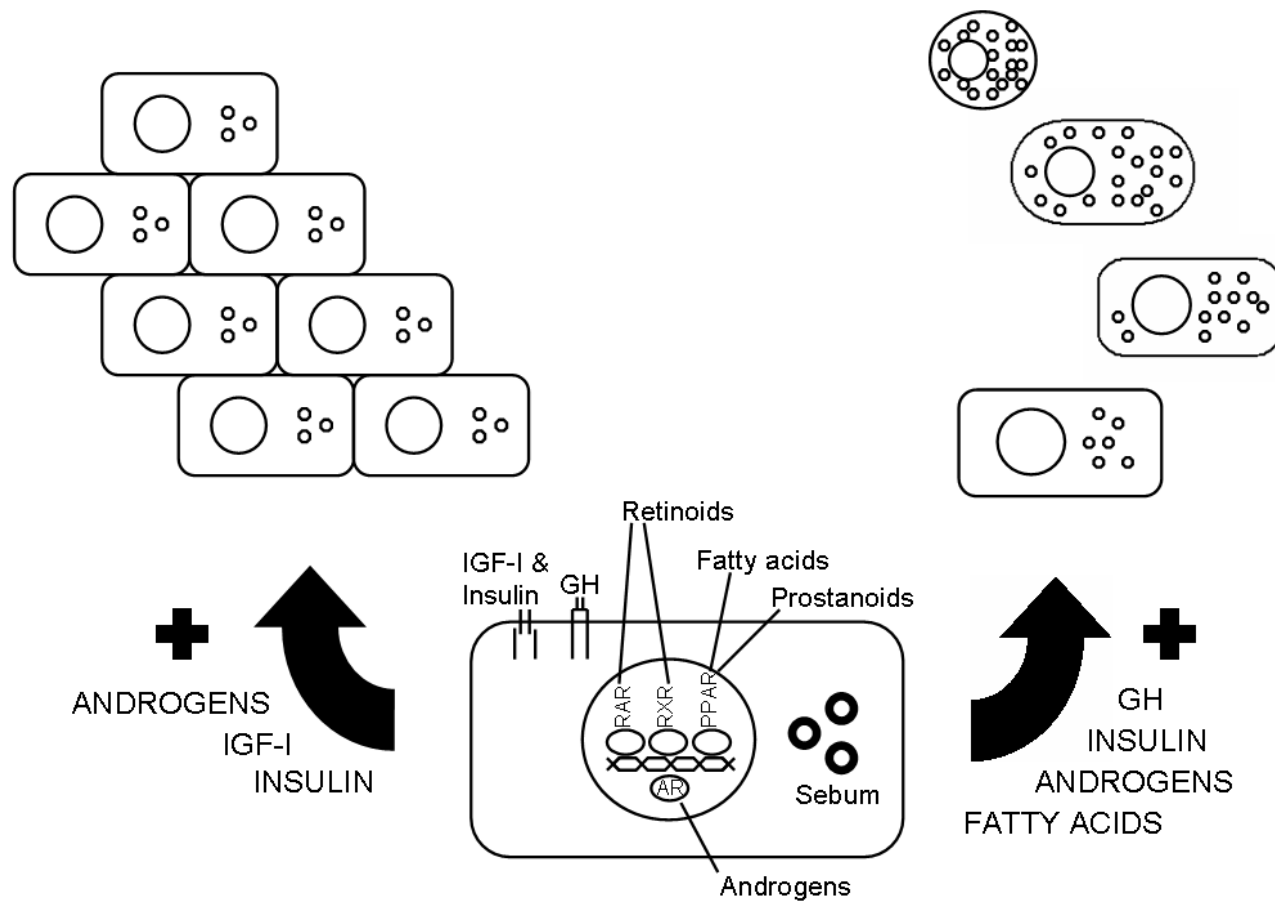


Figure 1.3: Important factors in cell proliferation and differentiation of sebocytes. Several biochemical factors are important for the proliferation and differentiation of sebocytes. Sebocyte growth is stimulated by androgens, IGF-I and insulin whereas differentiation is influenced by androgens, GH, insulin and fatty acids. Abnormal expression of one or more of these factors could lead to the development of sebaceous hypertrophy associated with acne.

1.7.1 Androgen production and bioavailability

Androgens, such as dehydroepiandrosterone (DHEA), androstenedione (A-dione) and testosterone, have a stimulatory effect on sebocyte differentiation and sebum production (Diamond *et al.*, 1996; Brown and Shalita, 1998; Fritsch *et al.*, 2001). However, patients with acne do not typically exhibit elevated serum levels of androgens (Gollnick *et al.*, 2003). Although elevated serum androgens correlate with cystic acne, androgen levels are normal in mild-moderate cases (Marynick *et al.*, 1983; Levell *et al.*, 1989). The principal androgen to exert biological effects is testosterone which may be locally converted to the more active 5 α -dihydrotestosterone (DHT) by 5 α -reductase (Gollnick, 2003). Acne has been shown to correlate better with serum levels of bioavailable testosterone, rather than to total testosterone. Patients with acne frequently demonstrate lower levels of SHBG and higher levels of free testosterone compared with controls (Olind *et al.*, 1982; Marynick *et al.*, 1983; Slayden *et al.*, 2001). It is thought that high testosterone levels, coupled with low SHBG levels, during puberty produces a maximum stimulatory effect on the sebaceous glands (Olind *et al.*, 1982). However post-puberty, in later teenage years, SHBG increases and testosterone levels plateau followed by general acne improvement (Olind *et al.*, 1982).

1.7.2 End-organ androgen metabolism

The peripheral conversion of androgen precursors to active androgens may also play a role in acne development. Androstenedione and DHEA-S are androgenic by means of their conversion to testosterone or DHT and sebocytes express all the steroidogenic enzymes required for their conversion (**Figure 1.4**) (Labrie *et al.*, 2000; Chen *et al.*, 2002). The androgenic precursors traverse the cell membrane of sebocytes where they can be converted to active androgens prior to binding to the androgen receptor (Barth and Clark, 2003). Although DHEA does not possess any intrinsic androgenic activity, plasma levels provide a large reservoir of substrate for its conversion in the peripheral intracrine tissues (i.e. sebaceous glands, hair follicles and sweat glands) (Labrie *et al.*, 2000). The term ‘intracrinology’ has been used to describe the process where action and synthesis occur within the same peripheral cell (Labrie, 1991). The intracrine actions of DHEA can increase sebum output, acne and/or some hair growth following the topical application or oral administration of DHEA (Diamond

et al., 1996; Morales *et al.*, 1998). Furthermore, lowering of elevated DHEA-S levels in patients with cystic acne results in remission of acne in most instances (Morales *et al.*, 1998).

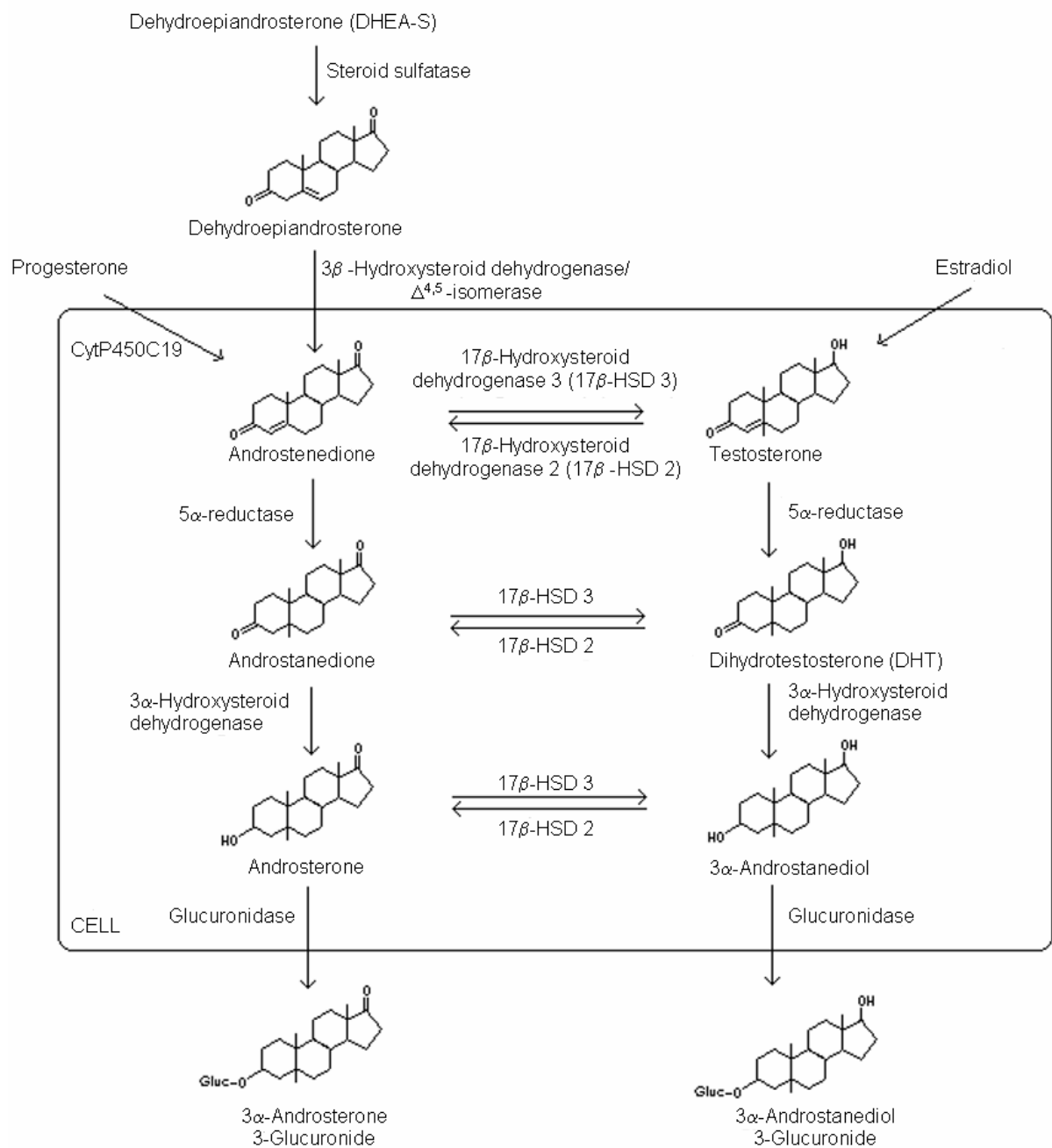


Figure 1.4: Pathways of cutaneous androgen metabolism in the sebaceous glands. Schematic representation illustrating the steroidogenic enzymes involved in the intracrine pathways. Weak androgens, such as DHEA-S, may be metabolised to form active hormones (testosterone and DHT), which exert their action in the same cells where synthesis took place (Adapted from Chen *et al*, 2002).

1.7.3 Growth hormone

Studies using the rodent preputial gland, which is comprised primarily of sebocytes, suggest that GH works in combination with androgens to stimulate gland activity (Laurent *et al.*, 1992). When castrated rats were given testosterone, the preputial glands became larger, but this effect was lost in hypopituitary-castrated rats, where GH production was limited (Ebling, 1957). The response to testosterone could only be fully restored when bovine GH was administered (Ebling *et al.*, 1975). This suggests that testosterone requires the presence of GH to exert its stimulatory effect. Furthermore, GH can stimulate sebocyte differentiation in a dose dependent fashion (Deplewski and Rosenfield, 1999). In humans, excess sebum output is a characteristic feature of acromegaly, a condition of elevated GH levels (Burton *et al.*, 1972). In contrast, individuals who are GH deficient have extremely low sebum secretion rates (Goolamali *et al.*, 1973).

1.7.4 Insulin like Growth Factor (IGF-I) and androgenic activity

IGF-I is a potent stimulator of sebocyte proliferation *in vitro*, but with little effect on sebocyte differentiation (Deplewski and Rosenfield, 1999). It has been localised in the highly mitotic basal cells of sebaceous preputial glands, with labelled IGF-I being detected in the periphery of the gland (Hansson *et al.*, 1988). Serum levels of IGF-I in women with postadolescent acne have been shown to be significantly higher than those of controls (Aizawa and Niimura, 1995). It is proposed that IGF-I may mediate some of the androgen effects on sebaceous glands by up-regulating enzymes responsible for local androgen metabolism (Horton *et al.*, 1993). IGF-I has been shown to stimulate 5 α -reductase activity (Horton *et al.*, 1993), but other groups were unable to confirm this association (Dykstra *et al.*, 1993).

1.7.5 Insulin and sebocyte differentiation

The effects of DHT and GH on sebocyte differentiation are severely attenuated when insulin is not present (Deplewski and Rosenfield, 1999). Insulin's potentiating affect on GH-induced sebocyte differentiation *in vitro* has been shown to be dose-related (Deplewski and Rosenfield, 1999). High doses of insulin are also capable of stimulating sebocyte proliferation *in vitro*. However, the authors proposed that insulin was acting as a surrogate for IGF-I at the observed supraphysiological dose

(Deplewski and Rosenfield, 1999). Both insulin and IGF-I may bind to either receptor, but with reduced affinity (Ullrich *et al.*, 1986).

A role of insulin in sebaceous gland lipid metabolism has been suggested following the phenotypic characterisation of skin of streptozotocin-induced diabetic mice (Sakai *et al.*, 2003). The diabetic mice exhibited a significant relative decrease in triglyceride content of the stratum corneum, together with an increase in wax/cholesterol esters, thus indicating changes in the lipid metabolism of sebaceous glands (Sakai *et al.*, 2003). Insulin is a known regulator of lipid biosynthetic enzymes and is required for normal differentiation of adipocytes (Geloan *et al.*, 1989). Adipocyte differentiation bears many similarities to sebocyte differentiation *in vitro*, suggesting that insulin plays a similar role in sebaceous lipogenesis (Jensen *et al.*, 1989).

1.8 FOLLICULAR DESQUAMATION AND FACTORS AFFECTING ABNORMAL KERATINISATION

The second important factor in acne development is follicular hyperkeratinisation and the subsequent obstruction of sebaceous follicles (Toyoda and Morohashi, 2001). A precursor acne lesion, otherwise known as a microcomedone, forms due to abnormalities in the desquamation of ductal keratinocytes. The follicle wall of a microcomedone is characterised by a thicker cornified layer, comprised of compacted keratinised cells, which appear larger than cells found in normal follicles (Knutson, 1974). This altered morphology suggests hyperproliferation of ductal corneocytes/keratinocytes and perhaps a premature terminal keratinisation process. Several markers of proliferation (ie. 3H-thymidine labelling and keratins 6 and 16) are increased in comedones compared to normal follicles (Hughes *et al.*, 1996). Several factors are thought to be involved in the process of comedogenesis, including sebaceous lipid composition, androgen concentrations, local cytokine production and the follicular bacterial population. Furthermore, histochemical studies have also shown that insulin and IGF-I and their receptors play fundamental roles in skin physiology.

1.8.1 Insulin and keratinocyte proliferation

Several lines of evidence suggest an active role of insulin in skin physiology. Extracellular lipids of the stratum corneum create the epidermal water barrier, a hydrophobic seal between the internal milieu and the outside environment (Rosenfield *et al.*, 2000). The epidermal barrier is vital for normal skin functioning and consists of a mixture of lipids that are synthesised in keratinocytes from two-carbon units (acetyl-CoA) sourced from carbohydrate catabolism (Elias, 1983; Swope *et al.*, 2001). Carbohydrate uptake is regulated primarily by extracellular insulin, which is delivered from the plasma *in vivo*.

Insulin is also an essential component for the growth and maturation of cultured skin substitutes which are used as adjunctive therapy for skin wounds (Swope *et al.*, 2001). In the presence of epidermal growth factor, insulin can stimulate the formation of several epithelial layers in culture, producing a stratum corneum similar to that of normal skin (Swope *et al.*, 2001). Higher concentrations of insulin can sustain keratinocyte proliferation for an extended period of time *in vitro* and it is thought that the glucose supply may be rate limiting to the stimulation of mitosis (Swope *et al.*, 2001).

Insulin resistance and its compensatory hyperinsulinaemia are frequently associated with proliferative tissue abnormalities of the skin (ie. acanthosis nigricans, skin tags). Insulin may be involved in the pathogenesis of these skin abnormalities despite resistance to its metabolic effects via the insulin receptor (Geffner and Golde, 1988). It is thought that these growth abnormalities may be due to insulin's ability to bind to the IGF-I receptor at high concentrations (Kahn *et al.*, 1976; Geffner and Golde, 1988; Neely *et al.*, 1991). Insulin can act as a surrogate for IGF-I as it has approximately 50% amino acid homology to the IGFs (Jones and Clemmons, 1995). Increased phosphorylation of the IGF-receptor occurs in proliferating and differentiating keratinocytes isolated from IR-KO mice compared with wild type mice (Wertheimer *et al.*, 2001). This suggests activation of the IGF-I receptor is increased to compensate for the lack of insulin receptor signalling.

1.8.2 *Insulin like Growth factor (IGF-I) and keratinocyte proliferation*

IGF-I is required to maintain structural and functional integrity of the skin, although its role is not completely understood. IGF-I appears to direct its effects towards cell mitogenesis and is necessary for basal proliferation of keratinocytes (Rudman *et al.*, 1997). The major source of circulating IGF-I is the liver, but IGF-I may also be produced by many cell types of the skin (eg. dermal fibroblasts, epidermal melanocytes and differentiated keratinocytes)(Tavakkol *et al.*, 1992; Rudman *et al.*, 1997). It is thought that IGF-I may serve as an autocrine/paracrine regulator of local tissue events. IGF-I receptors are expressed in basal epidermal keratinocytes, as well as in undifferentiated epithelial cells of the follicular infundibulum, sebaceous glands and the hair matrix (Hodak *et al.*, 1996). However, IGF-I receptor expression has been shown to extend beyond the basal layer in hyperproliferative skin disorders (Hodak *et al.*, 1996).

Excessive IGF-I production has been linked to hyperproliferative skin conditions such as skin tags, coarsening of facial features and acanthosis nigricans, similar to elevated insulin levels (Barkan *et al.*, 1988). Eming *et al.* (1996) showed that when human keratinocytes, genetically modified to overexpress IGF-I, were grafted onto athymic mice, hyperkeratosis and epidermal hyperplasia was observed (Eming *et al.*, 1996). Several days after grafting, epidermal structures were several cell layers thick and the cornified layer did not exfoliate, indicating that the thickness of the stratum corneum increased over time (Eming *et al.*, 1996). The modified epithelia showed increases in the expression of the proliferation markers (keratin 16 and Ki-67) (Eming *et al.*, 1996). IGF-I receptor knock-out mice show an abnormally thin and translucent epidermis with a reduced number of hair follicles (Liu *et al.*, 1993). Furthermore, IGF-I levels are decreased in the basal keratinocyte layer of sampled diabetic skin and that this could result in diminished proliferative activity of the keratinocytes (Blakytyn *et al.*, 2000).

1.8.3 *Androgens*

Androgens may also play a role in the follicular hyperkeratinisation seen in acne. Steroidogenic enzymes have been demonstrated in keratinocytes *in vitro*, thus indicating that these cells can produce active androgens via intracrine androgen metabolism (Gingras *et al.*, 2003). Furthermore, 5 α -

reductase activity is different in various anatomical areas of the skin, with infundibulum keratinocytes demonstrating greater 5α -reductase activity than the interfollicular epidermis (Thiboutot *et al.*, 1997). Comedonal acne correlates with DHEA-S levels before the onset of puberty (Lucky *et al.*, 1994; Lucky *et al.*, 1997), which suggests that the intracrine conversion of DHEA-S may be an important factor in follicular keratinisation.

1.8.4 Lipids

The prepubertal increase in sebum production is associated with significant changes in sebum composition (Stewart, 1992). Therefore, the critical factor may not be the amount of sebum, but rather the sebum composition. It has been suggested that follicles which are prone to acne have a relatively low concentration of linoleic acid. An examination of polar lipids from comedones revealed that acyl ceramides contain only 6% linoleate among the esterified fatty acids, compared with 45% in normal human epidermis (Wertz *et al.*, 1985). Furthermore, levels of linoleic acid normalise following treatment with oral isotretinoin or oral antiandrogens (Stewart *et al.*, 1986). It has been suggested that these changes may be due to dilutional effect of increasing lipid synthesis per sebocyte (Downing *et al.*, 1986). For example, the essential fatty acids, which are derived from the diet, may be diluted in the sebum of more active glands, compared to the less active glands. It is suggested that the resultant linoleate-deficient sebum bathes the follicular keratinocytes, resulting in disturbed keratinisation of the infundibulum and the formation of comedones (Downing *et al.*, 1987).

1.9 BACTERIAL COLONISATION

Bacterial colonisation of the sebaceous follicle with *Propionibacterium acnes* (*P.acnes*), is considered to be a major causative factor in the formation of inflammatory lesions. This anaerobic diphtheroid, increases by several orders of magnitude when sebaceous follicles become functionally blocked (Eady and Bojar, 2001). Therefore, *P.acnes* colonisation plays a secondary role in acne pathogenesis, as significant microbial involvement occurs only after sebum production has increased and comedone formation has become established (Bojar and Holland, 2004). *P.acnes* hydrolyses sebaceous triglycerides, consuming the glycerol fraction, while the liberated free fatty acids can have pro-inflammatory effects (Webster, 2002). The free fatty acids act as irritants and other chemotactic

factors released by bacteria can cause further damage and rupture of the follicular wall (Toyoda and Morohashi, 2001). Traditionally, antibiotics were used as a first-line therapy for acne, however increasing resistance of *P.acnes* in acne patients and the increasing pool of resistant organisms (including *Staphylococcus aureus*) has since limited their use (Gollnick *et al.*, 2003).

1.10 INFLAMMATION

The final pathogenic event in acne is the inflammatory reaction, which is triggered by a variety of pathological events. Inflamed lesions develop when comedones rupture and extrude their contents into the surrounding dermis. Bacteria can contribute to the inflammation by producing cytokines in a variety of different ways. Firstly, accumulation of the fatty acid waste products may promote the release of interleukin (IL) -1 α and tumour growth factor- α from keratinocytes lining the follicle wall (Downie *et al.*, 2002). Secondly, *P.acnes* and its cellular constituents may promote inflammation through the classical (involving antibodies) and alternative (naïve host) complement activation pathways, thus provoking the release of inflammatory cytokines (tumour necrosis factor (TNF)- α and interferon (IFN)- γ) (Downie *et al.*, 2002). And finally, *P.acnes* binds to the toll-like receptor on monocytes and neutrophils, leading to the production of multiple cytokines including IL-8, IL-12, and TNF- α . Hypersensitivity of the host immune response to *P.acnes* may also explain why some individuals are more prone to developing severe inflammatory acne.

A role for lipoxygenases in the pathogenesis of acne has recently provided evidence for a link between a dietary intake of pro-inflammatory omega-6 fatty acids and acne. A high omega-6/omega-3 dietary ratio can shift the eicosanoid balance in favour of metabolic products from arachidonic acid, specifically prostaglandins, thromboxanes and leukotrienes. Several metabolites of arachidonic acid (20:4n6), such as prostaglandin E₂ (PGE₂) 12-hydroxytetraenoic acid and leukotriene B₄ (LTB₄) are increased in cutaneous disorders, such as psoriasis and atopic dermatitis, and it has been suggested that these metabolites may be involved in disease pathogenesis through their pro-inflammatory effects (Bjørneboe *et al.*, 1988; Ziboh, 1989; Boelsma *et al.*, 2001). In acne, LTB₄ has been shown to induce recruitment and activation of neutrophils, monocytes and eosinophils (Zouboulis, 2001). Moreover,

LTB₄ has been shown to provoke the release of a number of pro-inflammatory cytokines. Treatment of acne with an anti-inflammatory agent, which specifically blocks the formation of LTB₄, has been shown to reduce inflammatory acne by approximately 70% (Zouboulis *et al.*, 2001). These patients also showed a decrease in total serum lipids, especially of pro-inflammatory lipids and this reduction was found to be directly correlated with the improvement of inflammatory lesions (Zouboulis *et al.*, 2001). Therefore, dietary supplementation with omega-3 fatty acids may help to decrease inflammatory acne through a reduction in the formation of LTB₄.

1.11 CONCLUSIONS

Current scientific opinion generally views diet as one of the many myths associated with acne. However, there is little experimental evidence in support of this widely held view. Furthermore, this view is based on a limited number of poorly controlled small studies which focused on individual foods and failed to examine the diet in its entirety. Given that acne involves of a complex etiology of several factors, it seems unlikely that one food could be responsible for its clinical manifestation. From our current understanding of nutrition, no single dietary element has been shown to be the cause of any other chronic disease. Evidence now suggests that virtually all Western lifestyle diseases have multifactorial dietary elements that underlie their etiology, and acne is unlikely to be an exception.

The diets of traditional societies have provided us with some insights into the possible origins of this commonplace disorder. Insulinotrophic dietary factors are pervasive in Western societies, but not in traditional cultures, and this may explain the absence of acne in these societies. Puberty is normally associated with a transient decrease in insulin sensitivity and as modern dietary trends, particularly amongst adolescents, favour high GI foods, this fall in insulin sensitivity may be accompanied by significant hyperinsulinaemia. This may accentuate the normal endocrine changes of puberty and play an underlying role in acne pathogenesis. Insulin has been shown to affect several aspects of puberty, including: sex steroid production, by amplifying GnRH secretion; SHBG production by the liver; adrenal androgen production; circulating IGF-I levels and IGFBP-1 production. Although causality has not been proven, the evidence is highly suggestive that these nutritionally-related factors may be involved in the underlying molecular and non-inflammatory mechanisms that lead to comedone

formation. Furthermore, the fatty acid composition of the diet may also influence the secondary inflammatory mechanisms that lead to the presentation of inflammatory lesions.

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CHAPTER TWO

A pilot study to determine the short term effects of a low glycaemic load diet on hormonal markers of acne

Robyn Smith¹, Neil Mann^{1,2}, Henna Mäkeläinen³, Jessica Roper¹, Anna Braue⁴,
George Varigos^{4,5}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Australian Technology Network, Centre for Metabolic Fitness

⁴Department of Biochemistry and Food Chemistry, Turku University, Finland

³Department of Dermatology, Royal Melbourne Hospital, Parkville, Australia

⁵Department of Dermatology, Royal Children's Hospital, Parkville, Australia

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2.1 ABSTRACT

Background: Observational evidence suggests that dietary glycaemic load may be one environmental factor contributing to the variation in acne prevalence worldwide.

Objective: To investigate the effect of a low glycaemic load (LGL) diet on endocrine aspects of *acne vulgaris*.

Methods: Twelve male acne sufferers (17.0 ± 0.4 yr) completed a parallel, controlled feeding trial involving a 7 day admission to a housing facility. Subjects consumed either a LGL diet (n=7; 25% energy from protein and 45% from carbohydrates) or a high glycaemic load (HGL) diet (n=5; 15% energy from protein, 55% energy from carbohydrate). Study outcomes included changes in the homeostasis model of insulin resistance (HOMA-IR), sex hormone binding globulin (SHBG), free androgen index (FAI), insulin-like growth factor-I (IGF-I) and its binding proteins (IGFBP-I and IGFBP-3).

Results: Changes in HOMA-IR were significantly different between groups at day 7 (-0.57 for LGL vs 0.14 for HGL, $P=0.03$). SHBG levels decreased significantly from baseline in the HGL group ($P=0.03$), while IGFBP-I and IGFBP-3 significantly increased ($P=0.03$ and $P=0.03$, respectively) in the LGL group.

Conclusion: These results suggest that increases in dietary glycaemic load may augment the biological activity of sex hormones and IGF-I, suggesting that HGL diets may aggravate potential factors involved in acne development.

2.2 INTRODUCTION

Acne pathogenesis is complex, with strong evidence supporting the involvement of increased sebum production, abnormal differentiation of skin keratinocytes, bacterial colonisation and inflammation (Thiboutot, 1997). Although scientific research has clarified many of the histological and immunological processes that characterise acne, the fundamental cause of the disease remains unknown. Experimental evidence has shown that androgen hormones are essential for acne development (Ebling, 1957; Pochi *et al.*, 1962); however the relationship between acne severity and circulating androgen levels has been difficult to prove. Variations in the clinical response to androgens suggests that acne development may also be affected by androgen bioavailability, androgenic precursors, and androgen receptor sensitivity (Shaw, 2002). Furthermore, acne development can also be influenced by other biological factors, including growth hormone, insulin-like growth factor (IGF-I), and insulin (Shaw, 2002).

It is possible that the expression of acne may be affected by endocrine changes which are closely related to the pubertal rise in insulin resistance. The insulin response to glucose loads is increased during normal puberty and adolescence, and insulin sensitivity is decreased compared with adults and prepubertal children (Bloch *et al.*, 1987; Moran *et al.*, 1999). Insulin can affect the entire androgen axis: the pituitary, where it acts as a gonadotrophin amplifier (Adashi *et al.*, 1981); the gonads where it stimulates androgen synthesis (Barbieri *et al.*, 1988; Bebakar *et al.*, 1990); the adrenal glands, where it stimulates production of androgenic precursors (Kristiansen *et al.*, 1997); and the liver, where it inhibits sex hormone binding globulin (SHBG) production (Holly *et al.*, 1989). Insulin can also have direct effects on sebum production (Deplewski and Rosenfield, 1999), and can amplify the growth promoting effects of IGF-I by inhibiting the production of IGF binding protein-1 (IGFBP-1) (Holly *et al.*, 1989). Therefore, it is possible that underlying changes in insulin metabolism may potentiate multiple factors involved in acne development.

Strong support for a link between acne and insulin resistance can be found in the high prevalence of acne in women with polycystic ovary syndrome (PCOS), a condition associated with insulin resistance, hyperinsulinaemia and hyperandrogenism (Dunaif *et al.*, 1989; Franks, 2003). Evidence is

accumulating that Western dietary habits contribute to this high risk hormonal profile (Carmina *et al.*, 2006), but the efficacy of dietary change in altering hormone levels has not been sufficiently investigated (Marsh and Brand-Miller, 2005). Treatments for PCOS now includes insulin-sensitising drugs or carbohydrate digestion inhibitors, which improve insulin sensitivity, restore fertility and alleviate acne (Ciotta *et al.*, 2001; Kazerooni and Dehghan-Kooshkghazi, 2003).

Dietary glycaemic load may be one environmental factor linking acne and hyperinsulinaemia. The glycaemic load may be interpreted as a measure of the blood glucose and insulin-raising potential of a meal or diet (Brand-Miller *et al.*, 2003). Unlike the glycaemic index (GI), which classifies the glycaemic response of carbohydrates on a gram-for-gram basis, the glycaemic load also takes into consideration the amount of carbohydrate consumed and is defined as the product of the GI and the carbohydrate content. Cordain *et al* (2001) recently proposed that a high glycaemic load Western diet may frequently expose adolescents to significant hyperinsulinaemia and a hormonal cascade that favours increased keratinocyte growth and androgen-mediated sebum production (Cordain *et al.*, 2002). This is supported by the observed low rates of acne among cultures existing on low glycaemic load (LGL) traditional diets, comprised of minimally processed plant foods or low amounts of carbohydrate (Verhagen *et al.*, 1968; Schaefer, 1971; Cordain *et al.*, 2002). Furthermore, when these societies urbanise and become exposed to a high glycaemic load (HGL) diet, acne becomes a common and sometimes severe problem (Schaefer, 1971). However, it remains unknown whether reducing dietary glycaemic load can alter clinical or endocrine aspects of acne. Therefore, in a controlled feeding trial, we investigated the short term effects of altering the dietary glycaemic load on endocrine variables associated with acne and insulin resistance.

2.3 METHODS

2.3.1 Study design

The experimental protocol followed a parallel, non-randomised feeding trial involving a 7 day admission to a temporarily-assigned research facility at Ballarat University (Ballarat, Victoria, Australia). The research facility consisted of two separate dormitories, each offering a fully equipped kitchen, dining area and accommodation for the overnight housing of subjects. In a well-controlled

feeding environment, seven subjects consumed a LGL diet and five consumed a HGL diet for a period of 7 days. To ensure optimal control of dietary interventions, subjects were housed and fed in separate wards according to their dietary group allocation. At days 0 and 7, a venous blood sample was taken after an overnight fast. In addition, capillary blood samples were obtained on day 3 every hour for the measurement of blood glucose responses to meals.

The primary endpoints of this study were changes in insulin sensitivity and hormonal markers of acne. This study was approved by the Human Ethics Committee of RMIT University and all subjects and guardians (if aged <18years) gave informed consent to participate in the study.

2.3.2 Study subjects

Twelve young males (aged 15-20yr) with mild-moderate acne were recruited from primary care physicians, dermatologists and newspaper advertisements. Participants were classified as having acne based on self-reported history of persistent acne (acne present on most days for the past 6 months). As several patients were recruited from specialists, this study was bound by a duty-of-care to provide non-harmful treatments to patients seeking medical treatment. It was considered unethical to place referred patients on an intervention that could potentially harm or worsen acne severity. For this reason, subjects exhibiting milder forms of the disease, who were not actively seeking treatment, were recruited as controls. This research was intended as a pilot study for the design of a larger, randomised dietary intervention trial.

2.3.3 Dietary intervention

Dietary glycaemic load was manipulated by means of modification to the amount and type of carbohydrate. The LGL diet was achieved by means of a reduction in the amount of carbohydrate and by utilising carbohydrates with a lower GI. To maintain energy intakes, the percentage of lost energy from carbohydrates was replaced with energy from protein. In contrast, the HGL diet supplied more energy from carbohydrates and less from protein, and included foods with a higher GI. The target macronutrient composition of the LGL diet was 25% energy from protein, 45% energy from carbohydrates and 30% energy from fats and that of the HGL diet was 15% energy from protein, 55%

energy from carbohydrates, and 30% energy from fats. For the average subject (age 17, height 177cm, weight 69kg), a 7 day menu plan was calculated to meet each diet's target macronutrient composition, as well as matching for energy (1.16MJ) and the composition of dietary fats (saturated 8%, polyunsaturated 7%, monounsaturated 13%) between groups. Main meals were prepared and served according to specifications in the planned menu, however subjects were allowed to eat *ad libitum* between meals. Foods were weighed before each meal, and any unconsumed portions were also weighed to determine the amount of food consumed. Subjects were instructed to only eat the foods provided or prepared within their housing ward during the 7 day trial period. Dietary intakes were calculated using Australia specific dietary analysis software (Foodworks, Xyris Software, Highgate Hill, Australia).

2.3.4 Calculation of dietary GI and glycaemic load

The dietary GI and glycaemic load were calculated using the following equations: dietary GI = $\Sigma(\text{GI for food item} \times \text{proportion of total carbohydrate contributed by item})$, and dietary glycaemic load = $\Sigma(\text{GI for food item} \times \text{its carbohydrate content in grams} \div 100)$. The GI values used had glucose as the reference food and were taken from reference tables (Foster-Powell *et al.*, 2002), and Sydney University's GI website (Sydney University, date unknown).

2.3.5 Diurnal measurement of capillary blood glucose

On day 3, blood glucose was measured every hour between 09:00-21:00 to verify differences in glycaemic load. Subjects were allowed to engage in light activities (eg. slow walking, easy physical work and sitting) during the day of testing, but were restricted from doing any moderate-high intensity activities. Capillary blood glucose was measured using the glucose dehydrogenase method (HemoCue 201+ glucose analyser, Sweden; intra-assay CV = 1.6%). The postprandial glucose responses were assessed using the incremental (iAUC) and total (tAUC) area under the glucose curve. iAUC and tAUC were geometrically calculated using the trapezoidal method (Wolever and Jenkins, 1986).

2.3.6 *Acne scoring*

Scaling of acne was performed by a dermatology registrar on day 0 of the study to provide information on subject characteristics at baseline. The registrar assessed facial acne occurrence and severity only, using the Cunliffe-Leeds lesion count technique (Burke and Cunliffe, 1984). Changes in acne severity were not reported as at study outcome due to the short duration of the study and as treatment response usually takes several weeks (Gollnick *et al.*, 2003).

2.3.7 *Laboratory analyses*

Code labelled serum samples were stored at -20°C for analysis post-study by an independent laboratory. Day 0 and day 7 samples for each subject were included in the same assay run to avoid inter-assay variability. Serum insulin was measured using a commercially available microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan; intra-assay CV: 4.0%). SHBG concentrations were assayed with a commercially available radioimmunoassay (Orion Diagnostica, Espoo, Finland; intra-assay CV = 2.5%). Total testosterone was measured using solid-phase radioimmunoassay (Diagnostic Products, Los Angeles, USA; intra-assay CV: 2.7%). The Free Androgen Index (FAI) was calculated as testosterone concentration (nmol/L) \times 100 \div SHBG concentration (nmol/L). Total IGF-I (intra-assay CV: 2.9 %) and dehydroepiandrosterone sulphate (DHEAS, intra-assay CV: 8.1%) were measured using semi-automated Immulite technology (Diagnostic Products, Los Angeles, USA). IGFBP-1 and IGFBP-3 were assayed with a non-commercial radioimmunoassay as previously described (Baxter and Martin, 1986; Baxter *et al.*, 1987). An automated Olympus analyser (Melville, USA) was used to measure total cholesterol, high density lipoprotein cholesterol and triglyceride levels (intra-assay CV were 1-2% for all tests). Low-density lipoprotein cholesterol was calculated by the Friedewald formula (Friedewald *et al.*, 1972).

2.3.8 *Insulin sensitivity*

The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate measure of insulin sensitivity. HOMA-IR was calculated as fasting glucose (mmol/L) \times insulin (μ U/mL) \div 22.5 (Matthews *et al.*, 1985).

2.3.9 Statistical analysis

All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois). Baseline data and nutritional characteristics were compared between groups using a Mann-Whitney U test or an independent-sample *t* test, depending upon whether or not the data was normally distributed. For the primary endpoints, general linear models were used to test for overall treatment differences with adjustments made for baseline data. Within group comparisons were performed using the paired *t* test. Changes from baseline are reported as adjusted means and percentages, with statistical analyses done for absolute values. *P*-values less than 0.05 were considered significant.

2.4 RESULTS

2.4.1 Subjects

Table 2.1 shows the baseline characteristics according to group allocation. The groups had similar baseline characteristics with the exception of acne lesion counts. The LGL group had a greater mean number of acne lesions than subjects on the HGL diet.

2.4.2 Dietary composition

Table 2.2 shows the composition of the mean baseline diet of all subjects and the LGL and HGL diets during the trial period. No significant group differences were observed for any dietary variables at baseline. During the trial, both diets were significantly different with regards to the dietary glycaemic load and dietary glycaemic index. Both diets were also consistent with their target macronutrient composition and the composition of dietary fats was comparable. Although subjects were permitted to eat *ad libitum* between meals, energy intake did not differ significantly between the groups.

Table 2.1: Baseline characteristics of subjects by dietary group

| Variable | LGL group (n=7) | HGL group (n=5) | <i>P</i> ¹ |
|--------------------------------------|-----------------|-----------------|-----------------------|
| Age (yr) | 16.6 ± 0.5 | 17.6 ± 0.5 | 0.18 |
| Acne lesion count | 34.7 ± 5.0 | 7.2 ± 2.5 | 0.001 |
| Weight (kg) | 65.4 ± 3.5 | 75.1 ± 9.3 | 0.37 |
| Body Mass Index (kg/m ²) | 20.5 ± 0.6 | 24.6 ± 2.2 | 0.15 |
| Waist circumference (cm) | 71.8 ± 2.4 | 81.5 ± 6.1 | 0.13 |
| Fasting glucose (mmol/L) | 4.66 ± 0.38 | 4.68 ± 0.24 | 0.94 |
| Fasting insulin (mU/L) | 7.91 ± 1.70 | 8.84 ± 2.01 | 0.73 |
| HOMA-IR index | 1.67 ± 0.39 | 1.82 ± 0.40 | 0.80 |
| IGF-I (nmol/L) | 42.4 ± 4.3 | 40.6 ± 5.1 | 0.80 |
| Testosterone (nmol/L) | 20.6 ± 2.1 | 16.6 ± 2.3 | 0.23 |
| SHBG (nmol/L) | 26.0 ± 4.0 | 19.4 ± 3.7 | 0.27 |

Data is expressed as means ± SEM

¹*P* value corresponds with an independent-sample t test or Mann-Whitney *U* test for means

Table 2.2: Dietary composition of the LGL and HGL diets at baseline and during the controlled feeding period

| Nutrient | Baseline ¹ | Trial period | | |
|------------------------------|-----------------------|-------------------------|---------------------------|----------------|
| | (n=13) | LGL (n=7) | HGL (n=5) | P ² |
| Energy, kJ/day | 9814 ± 542 | 10278 ± 912 | 11574 ± 425 ^c | 0.30 |
| Dietary glycaemic index | 58.1 ± 1.0 | 36.0 ± 0.3 ^c | 71.0 ± 1.4 ^c | <0.001 |
| Dietary glycaemic load | 175.7 ± 11.1 | 93.5 ± 9.2 ^c | 254.9 ± 10.6 ^d | <0.001 |
| Carbohydrate, % of total kJ | 52.1 ± 1.8 | 44.0 ± 1.0 ^c | 54.0 ± 2.4 | 0.001 |
| Protein, % of total kJ | 16.4 ± 1.0 | 24.1 ± 0.9 ^c | 15.6 ± 0.4 | <0.001 |
| Total fat, % of total kJ | 31.4 ± 1.2 | 31.0 ± 1.4 | 30.6 ± 1.8 | 0.85 |
| Fat subgroups, % of total kJ | | | | |
| Saturated | 13.2 ± 0.6 | 8.0 ± 0.3 ^d | 7.7 ± 0.4 ^d | 0.50 |
| Polyunsaturated | 4.1 ± 0.3 | 6.7 ± 0.7 ^c | 6.7 ± 0.4 ^e | 0.94 |
| Monounsaturated | 11.5 ± 0.6 | 13.6 ± 0.7 | 13.6 ± 0.9 | 0.96 |
| Cholesterol, mg/day | 296 ± 28 | 391 ± 17 ^c | 200 ± 9 | <0.001 |
| Dietary fibre, g/day | 19.3 ± 1.3 | 31.6 ± 3.9 | 22.5 ± 2.1 | 0.16 |

Data is expressed as means ± SEM

¹An independent-sample *t* test revealed no significant group differences at baseline.

²P value corresponds with independent-sample *t* test

Significant difference from baseline ^c(*P*<0.05), ^d(*P*<0.01), ^e(*P*<0.001)

2.4.3 *In vivo verification of dietary glycaemic load*

Figure 2.1 shows the postprandial blood glucose response to meals on day 3. Blood glucose rose to a higher peak in the HGL group after each meal. The calculated incremental area under the blood glucose curve (iAUC) was significantly greater for the HGL group compared to the LGL group ($P=0.02$; **Table 2.3**). However, the total calculated area under the curve was not significantly different between groups.

Tables 2.3 and 2.4 show the nutritional characteristics and foods consumed on the day of blood glucose monitoring. The diets were significantly different with regards to glycaemic load ($P<0.001$), dietary GI ($P=0.001$), percentage energy from carbohydrate ($P=0.01$) and percentage energy from protein ($P=0.02$). With regards to relationship of dietary variables and blood glucose profile, the iAUC correlated with dietary GI ($r=0.60$, $P=0.04$), but not glycaemic load ($P=0.08$).

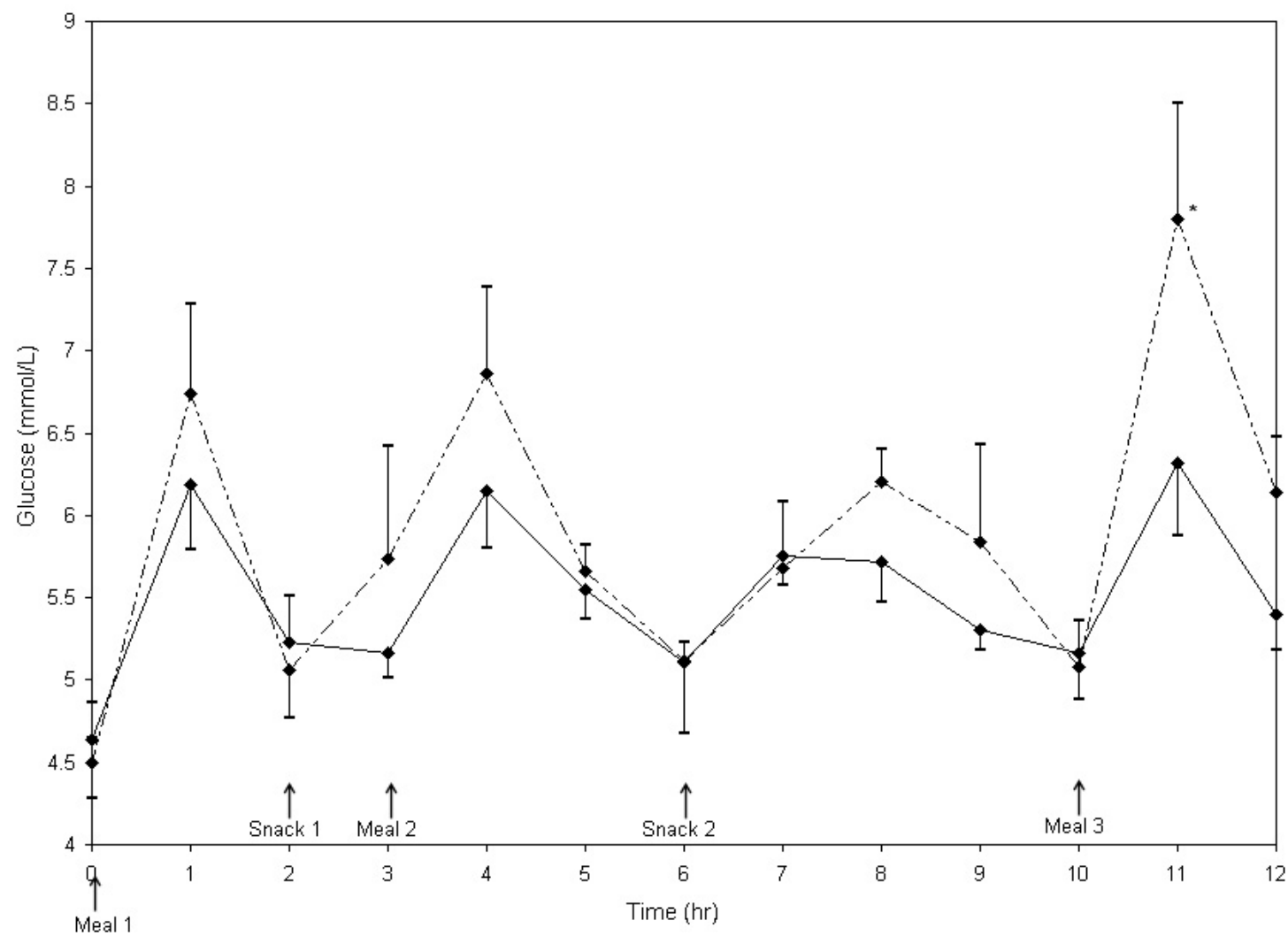


Figure 2.1: Capillary blood glucose profile for participants on the HGL or LGL diet

Values are mean \pm SEM. (—) LGL group ($n=6$); (---) HGL group ($n=5$).

Table 2.3: Nutritional characteristics and the area under the glucose curve for the day of diurnal blood glucose monitoring

| Variable | LGL group (n=7) | HGL group (n=5) | P |
|------------------------------------|-------------------------|-----------------|--------|
| Nutritional characteristics, Day 3 | | | |
| Energy, kJ | 9817 ± 660 | 10400 ± 568 | 0.54 |
| Dietary glycaemic load | 88.7 ± 6.7 | 248.7 ± 20.5 | <0.001 |
| Dietary glycaemic index | 33.1 ± 0.5 | 71.4 ± 1.4 | 0.001 |
| Carbohydrate, % of total kJ | 45.0 ± 2.5 | 56.9 ± 2.8 | 0.01 |
| Protein, % of total kJ | 22.8 ± 0.9 | 18.4 ± 1.5 | 0.02 |
| Fat, % of total kJ | 31.8 ± 3.0 | 24.2 ± 2.4 | 0.09 |
| Glycaemic profile, Day 3 | | | |
| iAUC, mmol·hr/L | 10.3 ± 1.5 [†] | 16.9 ± 1.9 | 0.02 |
| tAUC, mmol·hr/L | 66.4 ± 2.0 [†] | 70.9 ± 2.4 | 0.18 |

[†]n=6 due to an outlier of more than 3 standard deviations from the mean; subject has an undiagnosed myopathy.

Table 2.4: Experimental meals during the day of blood glucose monitoring

| LGL group (n=6) | | | HGL group(n=5) | |
|-----------------|-----------------------------|---------|-------------------------------|---------|
| Meal 1 | Bran based cereal or muesli | 40g | Corn or rice based cereal | 50g |
| | Milk, low-fat | 160g | Milk, skinny | 200g |
| | Bacon, fat removed, fried | 35g | Bread, white, increased fibre | 1 slice |
| | Egg, whole, fried | 35g | Honey/Jam | 1tsp |
| | Bread, mixed-grain | 2 slice | Margarine, polyunsaturated | 7g |
| | Margarine, canola | 7g | Fruit juice | 225ml |
| | Honey | 1tsp | | |
| | Apple juice | 225ml | | |
| Snack 1 | Yoghurt, low-fat | 200g | Yoghurt, low fat | 200g |
| | | | Lamington | 1 piece |
| Meal 2 | Bread, mixed grain | 4 slice | Bread, white, increased fibre | 4 slice |
| | Beef, silverside, lean | 150g | Ham, lean | 42g |
| | Salad, mixed | 60g | Cheese, low-fat | 45g |
| | Water | 500ml | Salad, mixed | 110g |
| | | | Water | 500ml |
| Snack 2 | Milk, low-fat | 200ml | Cordial beverage | 225ml |
| | Chocolate beverage base | 2 tsp | Cereal bar | 30g |
| | Dried fruit and nuts | 100g | | |
| Meal 3 | Beef, rump, lean, fried | 195g | Fish, poached | 150g |
| | Vegetables, raw | 170g | Potato chips, baked | 225g |
| | Honey | ½ tsp | Salad, raw | 80g |
| | Soy sauce, light | 1 ½ tsp | Fruit | 1 piece |
| | Noodles, mung bean, cooked | 75g | Ice cream, low-fat | 100g |
| | Frozen fruit dessert | 85g | Orange-flavoured soft drink | 225ml |

2.4.4 Study outcomes

Table 2.5 shows the mean change in hormonal variables and plasma lipids at day 7 according to dietary group. The mean change in fasting insulin levels ($P = 0.05$) and HOMA-IR ($P = 0.03$) was significantly different between groups with the LGL group showing a trend for improved insulin sensitivity and the HGL group showing a trend for increasing insulin resistance. At day 7, SHBG levels decreased by 9% ($P=0.03$) from baseline in the HGL group and the FAI increased by 19% ($P=0.03$). In contrast, no change in androgen levels or the FAI were observed in the LGL group. However, the LGL group demonstrated a 28% increase in IGFBP-1 levels ($P=0.03$) and a 27% increase in IGFBP-3 concentrations ($P=0.03$) when contrasted to the pre-treatment values. Both groups also demonstrated significant decreases from baseline in plasma total cholesterol ($P=0.004$ for LGL group and $P=0.03$ for HGL group) and LDL-cholesterol ($P=0.002$ for LGL group and $P=0.04$ for HGL group). In addition, the HGL group demonstrated a 10% decrease in HDL cholesterol ($P=0.01$), while the LGL group showed a 32% decrease in plasma triglycerides ($P<0.001$).

Table 2.5: Mean change in study endpoints after 7 days on experimental diets

| | Adjusted means ^a | | <i>P</i> ^b |
|--------------------------|-----------------------------|---------------------------|-----------------------|
| | LGL group (n=7) | HGL group (n=5) | |
| Fasting glucose (mmol/L) | -0.12 ± 0.11 | 0.10 ± 0.13 | 0.22 |
| Fasting insulin (mU/L) | -2.58 ± 0.88 | -0.59 ± 1.04 | 0.05 |
| HOMA-IR index | -0.57 ± 0.18 | 0.14 ± 0.22 | 0.03 |
| Testosterone (nmol/L) | -1.21 ± 0.76 | 1.00 ± 0.92 | 0.11 |
| SHBG (nmol/L) | -1.18 ± 0.75 | -2.14 ± 0.89 ^c | 0.44 |
| FAI (nmol/L) | -2.37 ± 5.91 | 19.39 ± 7.03 ^c | 0.04 |
| DHEA-S (μmol/L) | 0.32 ± 0.39 | -0.11 ± 0.46 | 0.50 |
| IGF-I (nmol/L) | -4.51 ± 1.95 | -7.23 ± 2.31 | 0.39 |
| IGFBP-1 (ng/L) | 8.65 ± 3.23 ^c | 0.38 ± 3.85 | 0.14 |
| IGFBP-3 (mg/L) | 0.73 ± 0.28 ^c | 0.23 ± 0.34 | 0.30 |
| T-Chol (mmol/L) | -0.81 ± 0.15 ^d | -0.63 ± 0.17 ^c | 0.45 |
| LDL-Chol (mmol/L) | -0.85 ± 0.14 ^d | -0.51 ± 0.16 ^c | 0.16 |
| HDL-Chol (mmol/L) | 0.03 ± 0.04 | -0.10 ± 0.05 ^d | 0.06 |
| Triglycerides (mmol/L) | -0.24 ± 0.34 ^e | 0.46 ± 0.41 | 0.25 |

^a Means are adjusted for baseline values^b General linear models were used to test for overall treatment differencesSignificant difference from baseline ^c(*P*<0.05), ^d(*P*<0.01), ^e(*P*<0.001)

2.5 DISCUSSION

This study showed that altering the dietary glycaemic load may have important effects on insulin sensitivity and factors related to acne. In a well-controlled feeding environment, subjects on the LGL diet demonstrated improvements in insulin sensitivity when compared to the HGL group. In addition, this study found that the HGL diet significantly increased androgen bioavailability; while increases in IGF-I binding proteins in the LGL group suggest a reduction in IGF-I activity. These results are concordant with the known relationship of insulin and the actions of androgens and IGF-I, and suggests that HGL diets may promote underlying causal factors associated with acne development.

The acute physiologic responses to feeding were also demonstrated in the marked between diet differences in day-long blood glucose responses. By maximising differences in glycaemic load, this study demonstrated a ~60% higher iAUC for glucose with the HGL diet when compared to the LGL diet. However, iAUC for glucose did not correlate with dietary glycaemic load possibly due to the small number of subjects and the frequency of blood glucose sampling in the early postprandial phase (<1hr). Instead, this study found that the calculated dietary GI predicted the day-long iAUC for glucose, suggesting that the GI of relative carbohydrate foods was an important determinant in postprandial glycaemia. This is consistent with previous mixed meal studies which found that blood glucose responses can be predicted from the weighted GI of constituent foods (Wolever and Jenkins, 1986; Chew *et al.*, 1988). Although the coingestion of protein and fat can reduce postprandial glycaemia (Nuttall *et al.*, 1984; Nuttall and Gannon, 1990), Wolever and Bolognesi (1996) observed that the amount and type of carbohydrate in mixed meals accounts for 90% of the variability in the blood glucose response. As seen in our study and others (Wolever and Jenkins, 1986; Chew *et al.*, 1988), variations in the protein and fat content of mixed meals did not appear to obscure the accumulative effect of component carbohydrates. This is supported by the observation that the glycaemic load can be used to predict day-long blood glucose and insulin responses for whole diets, independent of increases in protein consumption (Atkinson *et al.*, 2004).

One of the important findings of the present study was the rapid and significant effect of dietary intervention on fasting insulin and HOMA-IR. These findings are consistent with previous intervention studies which suggest that LGL diets can improve insulin sensitivity, independently of changes in caloric intake (Frost *et al.*, 1996; Frost *et al.*, 1998). However, clamping for total energy may limit the full benefit of LGL diets because of the influence of low GI foods on satiety and decreasing food intake (Ludwig *et al.*, 1999; Ball *et al.*, 2003). Accumulating evidence suggests that LGL interventions may facilitate weight loss in overweight and obese adolescents, without the need for an imposed energy restriction (Spieth *et al.*, 2000; Ebbeling *et al.*, 2003). Although both diets in the present study were essentially designed to prevent changes in body weight, the voluntary intake of food between meals allowed some degree of variability in energy consumption. However, we suspect that the difference in energy of the two diets was not sufficient in magnitude and/or duration to significantly impact on insulin resistance via changes in body fat distribution. Although the underlying mechanisms are yet to be fully defined, HGL diets may influence insulin sensitivity, independently of changes in weight or caloric intake, through elevations in: 1) plasma insulin (Del Prato *et al.*, 1994; Zammit *et al.*, 2001); 2) free fatty acids (Ludwig, 2002); 3) counterregulatory hormones (eg. cortisol and growth hormone); (Jenkins *et al.*, 1990) and 4) adipocyte diameter (Kabir *et al.*, 1997).

The results of the present study suggest that HGL diets may be associated with increases in androgen activity. This study found that the HGL diet increased androgen bioavailability by 19% and decreased SHBG levels by 9% when compared to pretreatment values. These changes may be partly explained by the ability of insulin to suppress SHBG synthesis at the hepatic level (Plymate *et al.*, 1998), thereby increasing the bioavailability of circulating androgens to tissues. In addition, insulin can further promote androgen bioavailability, by acting at the pituitary (Adashi *et al.*, 1981) and gonads (Barbieri *et al.*, 1988; Bebakar *et al.*, 1990) to increase androgen synthesis. These findings suggest that dietary glycaemic load may have important implications for acne and other clinical manifestation of androgen activity (eg. hirsutism, alopecia). Indirect support for this notion comes from evidence of the therapeutic effect of pharmacotherapies aimed at slowing the rate of carbohydrate absorption. Ciotta *et al.* (2001) recently demonstrated a 46% reduction in the acne/seborrhoea score in PCOS patients treated with an α -glucosidase inhibitor. The clinical improvement was associated with a significant

reduction in the insulin response to an oral glucose load, a decrease in androgen concentrations, with a significant rise in SHBG levels (Ciotta *et al.*, 2001). This suggests that reducing the postprandial rise in blood glucose concentrations per se may have a therapeutic effect on clinical and endocrine assessments of acne.

This study also suggests that LGL diets can influence IGF-I activity via changes in the level of IGF-binding proteins (IGFBPs). The LGL diet increased concentrations of IGFBP-1 by 28% and IGFBP-3 by 27% when compared to the pre-treatment values. These changes may be explained by the changes in the metabolic milieu associated with the consumption of a LGL diet. Insulin is the principle determinant of plasma IGFBP-1 levels and low basal IGFBP-1 levels have been observed in insulin resistant individuals, possibly due to increased portal insulin overnight (Attia *et al.*, 1998). IGFBP-3, on the other hand, may be affected by postprandial hyperglycemia as demonstrated by the significant reduction in IGFBP-3 concentrations after the consumption of a high GI meal when compared to a low GI meal (Brand-Miller *et al.*, 2005). Given that IGF-I is a potent stimulator of sebocyte (Deplewski and Rosenfield, 1999) and keratinocyte (Hodak *et al.*, 1996) proliferation, it is possible that LGL diets may induce changes to the IGF-I system which may be clinically relevant to acne development.

Several methodological issues pertaining to this study warrant further consideration. Firstly, as participants were not formally randomised to groups (for reasons previously outlined), we cannot underestimate the influence of selection bias. Nevertheless, our results are consistent with other randomised trials which report that LGL diets reduce androgen bioavailability and increase SHBG levels, while improving insulin metabolism (Berrino *et al.*, 2001; Smith *et al.*, 2007). The use of HOMA-IR as a surrogate measure of insulin sensitivity also warrants consideration. This index reflects hepatic insulin sensitivity and is based on the assumption that insulin sensitivity of the liver and peripheral tissues are equivalent (Monzillo and Hamdy, 2003). Although this index has been found to correlate with clamp-derived insulin sensitivity in large studies, its utility in small intervention trials remains uncertain. Furthermore, given the nature of the dietary intervention in this study, it is possible that the compensatory changes in other nutrients (eg. increased meat intake, micronutrients etc) may have influenced study endpoint measurements.

On the other hand, this study also had several strengths, including the precise control in the composition of foods provided and the accurate recording of dietary intakes. As participants were absolved from outside influences, dietary compliance could be achieved with minimal deviation from the dietary protocol. In nutrition research, knowledge of the level of dietary adherence is crucial when assessing the impact of dietary manipulations on physiologic processes (Hall and Most, 2005). By eliminating the variability associated with food selection and discrepancies in dietary compliance, this study was able to be performed with fewer numbers and under a shorter time frame.

In summary, these results suggest that LGL diets may have a therapeutic potential in the treatment of acne because of the beneficial endocrine effects of these diets. When compared to a typical HGL Western diet, a LGL diet was shown to reduce postprandial glycemia, while improving insulin sensitivity and decreasing androgen bioavailability. However, as this study only evaluated the short term effects of dietary intervention under ideal conditions, these suggestive findings will need to be substantiated by larger-scale experiments carried out under real life conditions.

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CHAPTER THREE

A low glycaemic load diet improves symptoms in *acne vulgaris* patients

A randomised controlled trial

Robyn N. Smith¹, Neil J. Mann^{1,2}, Anna Braue³, Henna Mäkeläinen⁴, George A. Varigos^{3,5}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Australian Technology Network, Centre for Metabolic Fitness

³Department of Dermatology, Royal Melbourne Hospital, Parkville, Australia

⁴Department of Biochemistry and Food Chemistry, Turku University, Finland

⁵Department of Dermatology, Royal Children's Hospital, Parkville, Australia

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3.1 ABSTRACT

Background: Although the pathogenesis of acne is currently unknown, recent epidemiologic studies of non-Westernised populations suggest that dietary factors, including the glycaemic load, may be involved.

Objective: The objective was to determine whether a low glycaemic load diet improves acne lesion counts in young males.

Design: Forty-three male acne patients aged 15-25yr were recruited for a 12-week, parallel design, dietary intervention incorporating investigator-blinded dermatology assessments. The experimental treatment was a low glycaemic load diet, comprised of 25% energy from protein and 45% from low glycaemic index carbohydrates. In contrast, the control situation emphasised carbohydrate-dense foods without reference to the glycaemic index. Acne lesion counts and severity were assessed during monthly visits and insulin sensitivity (using the homeostasis model assessment) was measured at baseline and 12 weeks.

Results: At 12 weeks, mean (\pm SEM) total lesion counts had decreased more ($P=0.03$) in the low glycaemic load group (-23.5 ± 3.9) than in the control group (-12.0 ± 3.5). The experimental diet also resulted in a greater reduction in weight (-2.9 ± 0.8 compared with 0.5 ± 0.3 kg; $P<0.001$), and body mass index (in kg/m^2 ; -0.92 ± 0.25 compared with 0.01 ± 0.11 kg/m^2 , $P=0.001$) and a greater improvement in insulin sensitivity (-0.22 ± 0.12 compared with 0.47 ± 0.31 , $P=0.02$) than did the control diet.

Conclusion: The improvement in acne and insulin sensitivity after a low glycaemic load diet suggests that nutrition-related lifestyle factors may play a role in the pathogenesis of acne. However, further studies are needed to isolate the independent effects of weight loss and dietary intervention and to further elucidate the underlying pathophysiologic mechanisms.

3.2 INTRODUCTION

Acne is a common and complex skin disease that affects individuals of all ages. In Western populations, it is estimated to affect 79% to 95% of the adolescent population, 40 to 54% of individuals older than 25 yr and 12% of women and 3% of men by middle age (Cordain *et al.*, 2002). In contrast, acne remains rare in non-Westernised societies such as the Inuit (Schaefer, 1971), Okinawan Islanders (Steiner, 1946), Ache hunter gatherers and Kitavan Islanders (Cordain *et al.*, 2002). Although familial and ethnic factors are implicated in acne prevalence, this observation is complicated by findings that acne incidence rates have increased with the adoption of Western lifestyles (Schaefer, 1971). These observations suggest that lifestyle factors, including diet, may be involved in acne pathogenesis.

Historically, much debate has surrounded the subject of diet in the management of acne. In the 1930s, acne was considered to be a disease of disturbed carbohydrate metabolism as early work suggested impaired glucose tolerance occurred in acne patients (Campbell, 1931). On the basis of these observations and the anecdotal impressions of physicians, patients were often discouraged from eating excessive amounts of carbohydrates and high-sugar foods (Sulzberger and Baer, 1948; Belsario, 1951). The diet and acne connection finally fell from favour in 1969 when a clinical study found no exacerbation of acne lesions in a group that ingested a chocolate bar compared with a group that ingested a placebo bar (Fulton *et al.*, 1969). Although it is the most widely cited reference dissociating diet and acne, this study has been criticised for a number of design flaws, including the similar nutrient composition of the placebo (Mackie and Mackie, 1974; Rasmussen, 1977; Cordain, 2005).

Recently, there has been a reappraisal of the diet and acne connection because of a greater understanding of how diet may affect endocrine factors involved in acne (Cordain *et al.*, 2002; Cordain, 2005). Of interest is the concept of the glycaemic index (GI) - a system of classifying the glycaemic response of carbohydrates. Because the GI can only be used to compare foods of equal carbohydrate content, the glycaemic load was later developed to characterise the glycaemic effect of whole meals or diets ($\text{GI} \times \text{available dietary carbohydrate}$). Cordain *et al.* (2002) postulated that high glycaemic load diets may be a significant contributor to the high prevalence of acne seen in Western

countries. The authors speculate that the frequent consumption of high-GI carbohydrates may repeatedly expose adolescents to acute hyperinsulinaemia. Hyperinsulinaemia has been implicated in acne pathophysiology because of its association with increased androgen bioavailability and free levels of insulin-like growth factor I (IGF-I) (Cordain *et al.*, 2003; Cordain, 2005). Therefore, we hypothesised that low glycaemic load dietary interventions may have a therapeutic effect on acne based on the beneficial endocrine effects of these diets. Consequently, the aim of this preliminary study was to investigate the efficacy of a low glycaemic load diet in reducing the severity of acne symptoms.

3.3 SUBJECTS

Males with facial acne were recruited through posted fliers at the RMIT University (Melbourne, Australia) and newspaper advertisements. Informed consent was obtained from each participant or guardian (if aged <18yr), and the study was conducted at RMIT University after obtaining approval from the RMIT Human Ethics committee. This study included only male participants, aged 15-25yr with mild to moderate facial acne. Participants were required to have had acne for longer than 6 months prior to recruitment. Individuals were excluded if they were currently taking medications known to affect acne or glucose metabolism. Additionally, a wash-out period of 6 months was required for subjects who had previously taken oral retinoids or 2 months for subjects who had taken oral antibiotics or topical antibacterial or retinoid agents.

3.4 METHODS

3.4.1 Study Design

It was calculated that 19 subjects per group would provide 80% power (at the 2-sided 5% level) to detect a difference of 20% in the reduction of acne lesions between groups, assuming a standard deviation of 22%. To compensate for subject withdrawal, 54 subjects were enrolled in the study.

Eligible participants were recruited between June 2003 and June 2004. Approximately 2-3 weeks after recruitment, participants attended their baseline appointment and were randomly assigned to either the

low glycaemic load (LGL) or control group (**Figure 3.1**). Randomisation was carried out by computer generated random numbers and allocation to groups was performed by a third party.

This study was designed as a parallel dietary intervention study with investigator-blinded dermatology assessments. Topical therapy, in the form of a noncomedogenic cleanser, was standardised for both groups and facial acne was scored at monthly visits (weeks 0, 4, 8, and 12) at the academic research clinic. On all visits, height and weight were measured. All subjects were weighed in light clothes and body mass index (BMI) was calculated as weight (kg)/height (m) squared. At baseline and 12 weeks, a venous blood sample was taken after an overnight fast and an oral glucose insulin sensitivity test was performed on a subgroup of participants from the LGL and control groups.

The primary endpoints of the study were changes in inflammatory lesion counts (papules, pustules and nodules) and total lesion counts (inflammatory lesions and non-inflammatory lesions) after 12 weeks. Secondary endpoints include changes in anthropometric measures and insulin sensitivity indexes.

3.4.2 Dietary intervention

Participants were informed that the study's intent was to compare the dietary carbohydrate to protein ratio and were not informed of the study's true intent. The LGL diet was achieved through modifications of the amount and type of carbohydrate. The LGL group was instructed to substitute high GI foods with foods higher in protein (e.g. lean meat, poultry or fish) or lower in GI (e.g. whole grain bread, pasta, fruit). Some staple foods were supplied and participants were urged to consume these or similar foods daily. Each participant received individualised dietary plans that were isocalorically matched with their baseline diet as determined from 7-day weighed/measured food records. The recommended LGL diet consisted of 25% energy from protein, 45% from low GI carbohydrates and 30% energy from fats. In contrast, the control group received carbohydrate-dense staples and were instructed to eat these or similar foods daily. The foods provided had moderate-to-high GI values and were typical of their normal diet as evidenced from 7-day weighed and measured food records. The control group were not informed about the GI, but were urged to include carbohydrates as a regular part of their diet. All participants received initial education on use of foods

scales and keeping of foods records. During the study period, nutrient intakes were calculated from 3-day weighed and measured food records each month using Australia specific dietary analysis software (Foodworks, Xyris Software, Highgate Hill, Australia). Dietary compliance was monitored by regular telephone interviews, assessments of GL /day and 24-hr urine samples (weeks 0 and 12) for an assessment of urea excretion relative to urinary creatinine as a marker of protein intake.

3.4.3 Calculation of dietary glycaemic index and glycaemic load

Daily dietary glycaemic index and glycaemic load were calculated from diet records. The dietary GI and glycaemic load were calculated using the following equations: dietary GI= $\Sigma(\text{GI for food item} \times \text{proportion of total carbohydrate contributed by item})$, and dietary glycaemic load = $\Sigma(\text{GI for food item} \times \text{its carbohydrate content in grams} \div 100)$. The GI values used had glucose as the reference food and were taken from reference tables (Foster-Powell *et al.*, 2002) and Sydney University's GI website (Sydney University, date unknown). If a food from Australia was not available, the GI was estimated by using similar foods of known value.

3.4.4 Standardised topical lotion

All participants were provided with a topical cleanser (Cetaphil gentle skin cleanser, Galderma, Frenchs Forrest, Australia) and advised to use it in place of their normal wash, soap or cleanser. Subjects began using the topical wash 2 weeks prior to baseline and were asked to maintain a standard level of usage during the trial.

3.4.5 Dermatology assessment

Scaling of the acne was performed by a dermatology registrar who was blinded to the group assignment of the participants. The registrar assessed facial acne occurrence and severity only, using a modified Cunliffe-Leeds lesion count technique (Burke and Cunliffe, 1984). To ensure all acne lesions were counted, located and graded by size and severity, lesions were mapped by placing a transparent plastic film with a laser-printed grid gently against the skin. Facial anatomical landmarks, such as the ear, chin and tip of nose, were used to ensure consistency between assessments. Each side of the face

was assessed separately. Where necessary, the registrar palpated the skin to determine the lesion type. To maintain reproducibility of this procedure, one physician performed all the dermatology assessments. A small group of volunteers (n=4) was counted one-week apart to evaluate the reproducibility by the same physician [9.5% coefficient of variation (CV)].

3.4.5 Laboratory analyses

Code labelled serum samples were stored at -80°C for analysis post-study by an independent laboratory. Baseline and 12-week samples for each participant were included in the same assay run to avoid inter-assay variability. Serum insulin was measured using a commercially available microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan; intra-assay CV: 4.0%). Capillary blood glucose was measured on the day of testing using a Glucose 201+ analyser (HemoCue, Sweden; intra-assay CV = 1.6%).

3.4.6 Insulin sensitivity measures

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: fasting glucose (mmol/L) \times fasting insulin (μ U/mL) \div 22.5 (Matthews *et al.*, 1985). We also used the model-derived formula proposed by Mari *et al* (Mari *et al.*, 2001) to calculate the oral glucose insulin sensitivity (OGIS) index from a 2hr oral glucose tolerance test. This formula was calculated using six fixed rate constants, the oral glucose dose (75g); body surface area; glucose concentrations (mg/dL) at 0, 90, and 120 min; and insulin concentrations (μ U/mL) at 0 and 90min. In the present study, some participants were unable to sit the 2hr test due to scheduling interference (eg. school, work commitments). Consequently, the OGIS data presented are for only 18 subjects in the LGL group and 17 subjects in the control group.

3.4.7 Statistical analysis

All statistical analyses were performed with the use of SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois). Baseline characteristics were analysed for between group significance using Mann-Whitney U test or an independent-sample *t* test, depending upon whether or not the data was normally

distributed. Repeated-measures analysis of variance was used to analyse dietary and acne (lesion count) data and to explore the effects of time, treatment and an interaction of these two factors. We compared changes in lesion counts using repeated measures analysis of log-transformed data at each follow up visit with baseline lesion counts as the covariate. *P*-values less than 0.05 were considered significant. Analysis of covariance (ANCOVA) was used to test for overall treatment differences at 12-weeks, with baseline data as the covariate. Secondary analyses were performed with adjustment for changes in BMI.

The primary clinical outcome (changes in lesion counts at 12 weeks) was analysed using an intention-to-treat model for all randomised subjects using the last measurement carried forward for all missing data.

Bivariate linear regression analysis was also conducted, pooling data from both groups, to explore relationships between dietary variables and acne improvement.

3.5 RESULTS

3.5.1 Subjects

Forty-three subjects completed the study per protocol (**Figure 3.1**). Seven participants did not complete the study (five in control and two in LGL) and four were removed from data set (two began acne medications and two were non-compliant). Baseline characteristics are shown in **Table 3.1**.

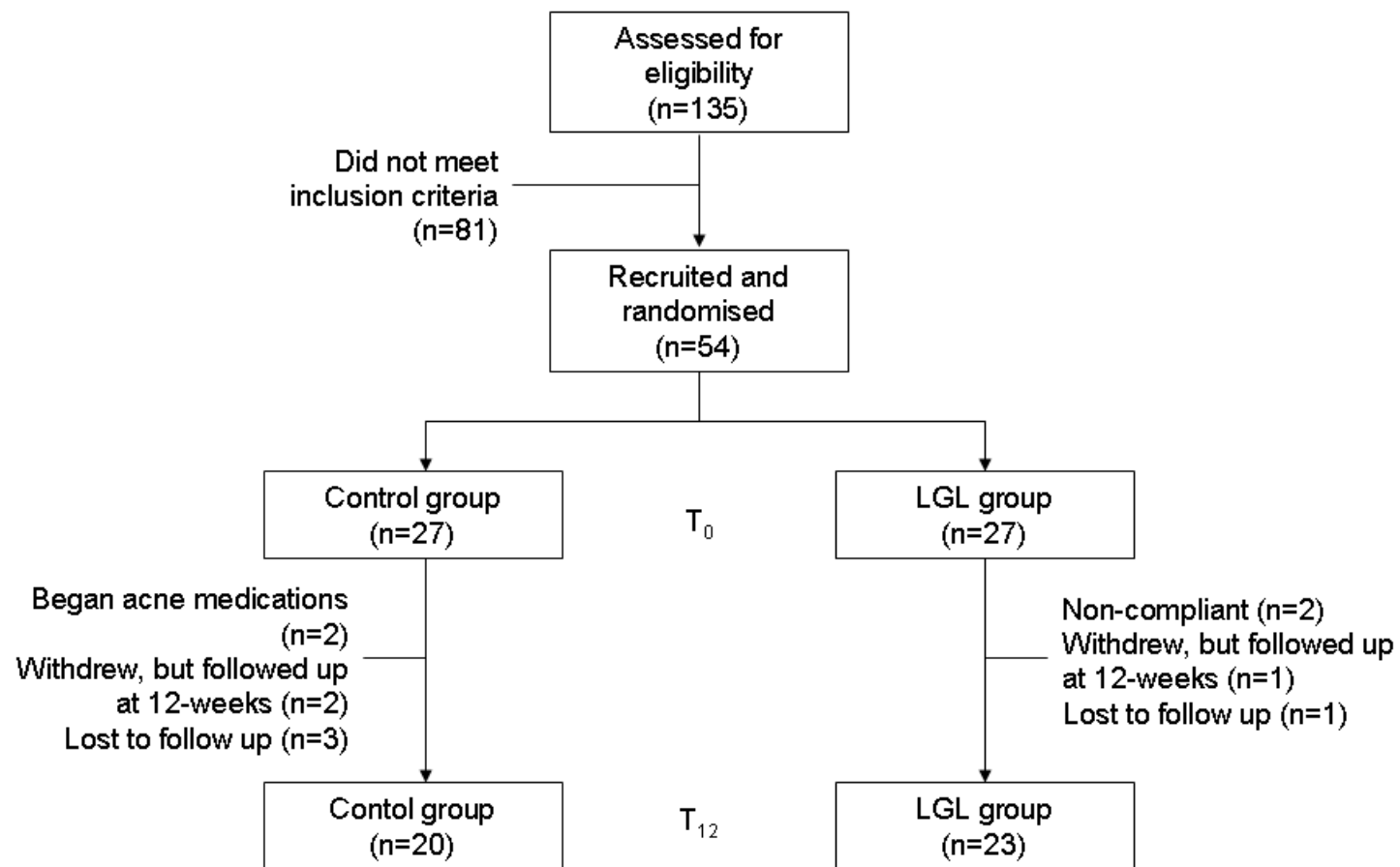


Figure 3.1: Recruitment to completion of participants after 12 weeks (T_0 =baseline, T_{12} =12 weeks)

Table 3.1: Subject characteristics at baseline by dietary group

| Variable | LGL group (<i>n</i> = 23) | CO group (<i>n</i> = 20) | <i>P</i> ^{<i>I</i>} |
|--|-------------------------------|------------------------------|------------------------------|
| Ethnicity (<i>n</i>) | | | |
| Caucasian | 20 | 17 | 0.60 |
| Asian | 3 | 3 | |
| Age (yr) | 18.2 ± 0.5 ² | 18.5 ± 0.5 | 0.76 |
| Body weight (kg) | 73.5 ± 2.5 | 73.3 ± 3.3 | 0.90 |
| Body Mass Index (kg/m ²) | 22.9 ± 0.6 | 22.5 ± 0.7 | 0.34 |
| Total lesion count | 40.6 ± 5.0 | 34.9 ± 4.3 | 0.40 |
| Inflammatory lesion count | 31.9 ± 3.9 | 28.4 ± 3.6 | 0.72 |
| OGIS (mL·m ⁻² ·min ⁻¹) ³ | 481 ± 9 | 503 ± 9 | 0.12 |
| HOMA-IR | 1.6 ± 0.2 | 1.5 ± 0.2 | 0.88 |

^{*I*} Corresponds with an independent-sample *t* test or Mann-Whitney *U* test for means and the Fisher's exact test for ethnicity

² Data is expressed as means ± SEM (for all such values).

³ *n*=18 for the LGL group and *n*=17 for the control group

3.5.2 *Diet composition*

Dietary intakes of the LGL and control groups at baseline and during the trial is shown in **Table 3.2**. No significant group differences were observed in any of the dietary variables at baseline. During the trial period, dietary glycaemic load decreased significantly in the LGL group compared with the control group, and this change was achieved by a reduction in carbohydrate intake and by inclusion of low-GI foods (as indicated by a reduction in the calculated dietary GI). Protein intake increased in the LGL and decreased slightly in the control group, indicating that some carbohydrates were replaced with foods higher in protein. This observation was substantiated by a 15.4% increase in urinary urea to creatinine ratio at 12 weeks for the LGL group compared with a 12.3% decrease for the control group ($P=0.009$), which indicated good dietary compliance.

Table 3.2: Dietary intakes of low glycaemic load (LGL) and control groups at baseline and during the trial period

| Variable | LGL group (n=23) | Control group (n=20) | P^1 | | |
|-----------------------------|------------------------------|-------------------------|--------|--------|------------------------------------|
| | | | Group | Time | Group \times time interaction |
| Energy (kJ/day) | | | 0.15 | 0.09 | 0.100 |
| Baseline ² | 10625 \pm 572 ³ | 10540 \pm 546 | | | |
| Trial period ⁴ | 9320 \pm 460 | 10620 \pm 494 | | | |
| Dietary glycaemic index | | | <0.001 | <0.001 | <0.001 |
| Baseline | 57.5 \pm 1.0 | 57.2 \pm 0.8 | | | |
| Trial period | 43.2 \pm 0.8 | 56.4 \pm 0.8 | | | |
| Dietary glycaemic load | | | <0.001 | <0.001 | <0.001 |
| Baseline | 174.7 \pm 9.1 | 181.5 \pm 11.5 | | | |
| Trial period | 101.5 \pm 6.1 | 174.3 \pm 10.7 | | | |
| Carbohydrate, % of total kJ | | | 0.013 | 0.195 | 0.001 |
| Baseline | 50.2 \pm 1.1 | 48.2 \pm 1.2 | | | |
| Trial period | 44.1 \pm 1.3 | 50.1 \pm 1.2 | | | |
| Protein, % total kJ | | | 0.001 | <0.001 | <0.001 |
| Baseline | 16.3 \pm 0.6 | 17.5 \pm 0.9 | | | |
| Trial period | 22.7 \pm 0.8 | 17.4 \pm 0.8 | | | |
| Total fat, % total kJ | | | 0.84 | 0.74 | 0.45 |
| Baseline | 32.5 \pm 1.1 | 31.8 \pm 1.2 | | | |
| Trial period | 31.5 \pm 0.9 | 31.4 \pm 1.2 | | | |
| Saturated fat, % total kJ | | | 0.002 | <0.001 | <0.001 |
| Baseline | 13.5 \pm 0.6 | 12.9 \pm 0.7 | | | |
| Trial period | 9.0 \pm 0.4 | 13.0 \pm 0.9 | | | |
| Fibre, g/day | | | 0.002 | <0.001 | <0.001 |
| Baseline | 25.3 \pm 1.8 | 25.2 \pm 2.1 | | | |
| Trial period | 36.9 \pm 2.0 | 25.2 \pm 2.0 | | | |

¹ Repeated measures ANOVA was done to incorporate data from all time points and to evaluate the differences between the LGL and control groups (main effect of group), the change over time (main effect of time) and the differences in the time course between the two groups (group \times time interaction).

² An independent-sample *t* test revealed no significant differences between the LGL and control groups for any of the listed dietary variables at baseline.

³ All data is expressed as mean \pm SEM

⁴ Means of data collected at 4, 8 and 12 weeks

3.5.3 *Study outcomes*

As shown in **Figure 3.2**, both the LGL and control groups showed reductions in inflammatory and total lesion counts from 0 to 12 weeks. Repeated-measures analysis that used baseline counts as the covariate indicated an overall difference between the LGL and control groups (main effect of group), but no significant change over time (main effect of time), or difference in the time course between the groups (group \times time interaction) was observed. At 12 weeks, the LGL group had a greater reduction in the mean number of total and inflammatory lesions than did the control group (**Table 3.3**). Examples of acne improvement in the LGL group are shown in **Figure 3.3**. The mean number of total lesions fell by 23.5 (51%) in the LGL group and by 12.0 (31%) in the CO group ($P=0.03$). Inflammatory lesions counts fell by 17.0 (45%) in the LGL group and by 7.4 (23%) in the control group ($P=0.02$). The results at 12 weeks were also materially unchanged by intention-to-treat analysis.

The LGL group also showed significant reductions in weight ($P<0.001$), BMI ($P=0.001$) and HOMA-IR ($P=0.026$) when compared to the control group. The change in HOMA-IR correlated with the change in OGIS index ($r=-0.36$, $P=0.035$), with both models suggesting a trend for improved insulin sensitivity in the LGL group and a trend for increasing insulin resistance in the control group. Statistical adjustment of study endpoints for the change in BMI was found to alter the outcome for HOMA-IR ($P=0.10$) and total lesion counts ($P=0.07$), but not inflammatory counts ($P=0.04$). However, we found no significant interaction effect of dietary treatment and the change in BMI on acne lesion counts.

3.5.4 *Dietary variables as predictors of acne improvement*

Dietary correlates with acne improvement include reductions in glycaemic load ($r = 0.49$, $P = 0.001$), dietary GI ($r = 0.30$, $P = 0.05$), carbohydrate intake ($r = 0.46$, $P = 0.002$), percentage saturated fat ($r = 0.36$, $P = 0.02$) and total energy intake ($r = 0.40$, $P = 0.01$). These relationships are shown in **Figures 3.4**.

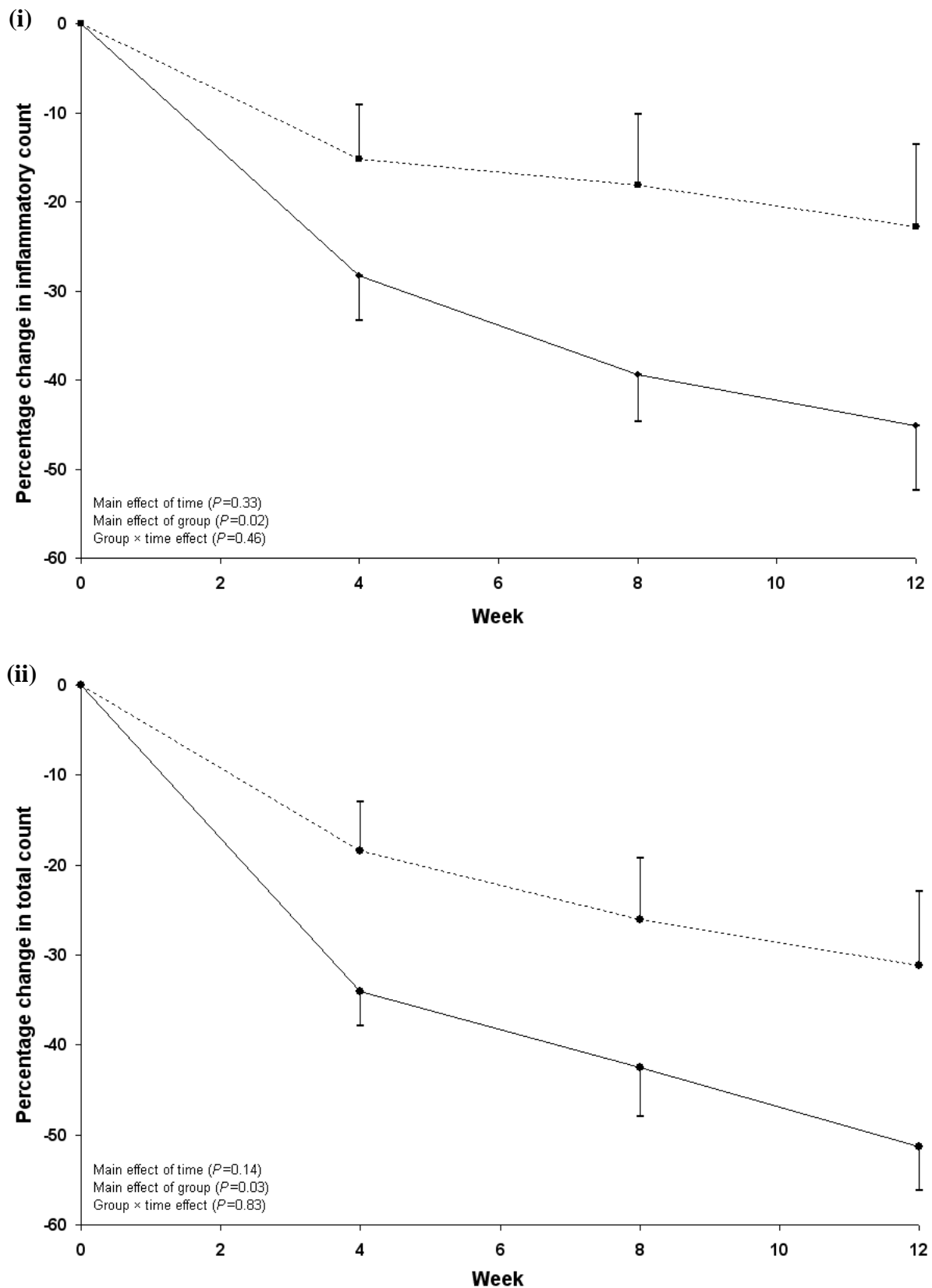


Figure 3.2: Mean (\pm SEM) percentage changes from baseline in inflammatory acne lesion counts and in total acne lesion counts for the low glycaemic load group (solid line; $n=23$) and the control group (dashed line; $n=20$) at each visit. Repeated-measures ANOVA was performed incorporating the absolute data (log transformed) from each follow up visit, with baseline counts as the covariate.

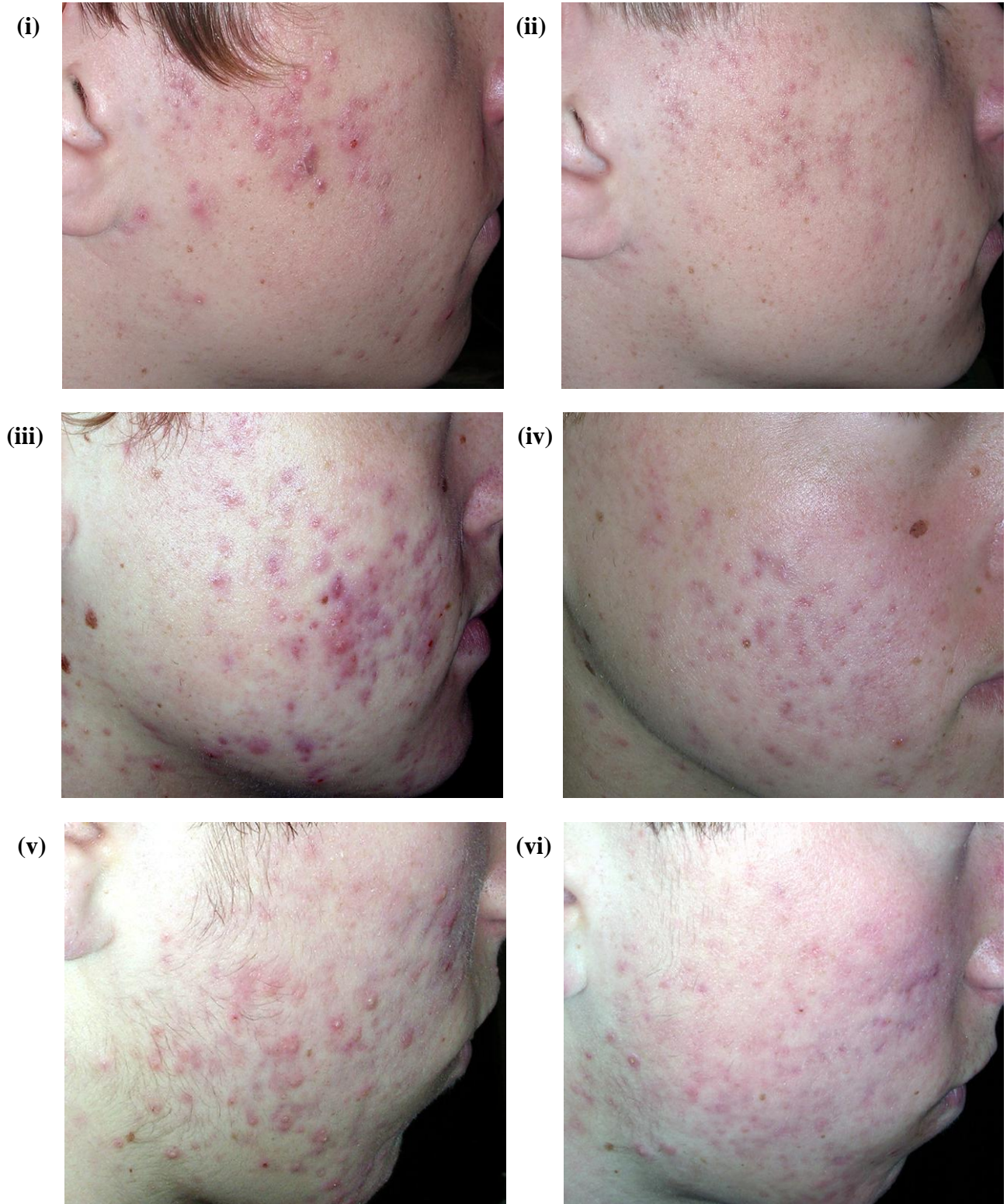
Table 3.3: Absolute mean differences from baseline to 12 weeks in outcome variables by dietary group

| Variable | LGL group | | CO group | | P^I |
|---|--------------------|----|-----------------|----|--------|
| | Difference | n | Difference | n | |
| <i>Per-protocol analysis</i> | | | | | |
| Total count | -23.5 ± 3.92^2 | 23 | -12.0 ± 3.5 | 20 | 0.03 |
| Inflammatory count | -17.0 ± 3.1 | 23 | -7.4 ± 2.5 | 20 | 0.02 |
| Weight (kg) | -2.9 ± 0.8 | 23 | 0.5 ± 0.3 | 20 | <0.001 |
| BMI (kg/m ²) | -0.92 ± 0.25 | 23 | 0.01 ± 0.11 | 20 | 0.001 |
| HOMA-IR ³ | -0.22 ± 0.12 | 23 | 0.47 ± 0.31 | 20 | 0.03 |
| OGIS (mL·m ⁻² ·min ⁻¹) | 12.7 ± 7.9 | 16 | -18.3 ± 9.9 | 17 | 0.08 |
| <i>Intention-to-treat analysis</i> | | | | | |
| Total count | -22.0 ± 3.5 | 27 | -10.9 ± 2.9 | 27 | 0.02 |
| Inflammatory count | -16.2 ± 2.9 | 27 | -5.6 ± 2.5 | 27 | 0.01 |

¹ Testing using ANCOVA for between group comparison with baseline as a covariate

² All data is expressed as absolute mean difference (12 weeks minus baseline) \pm SEM

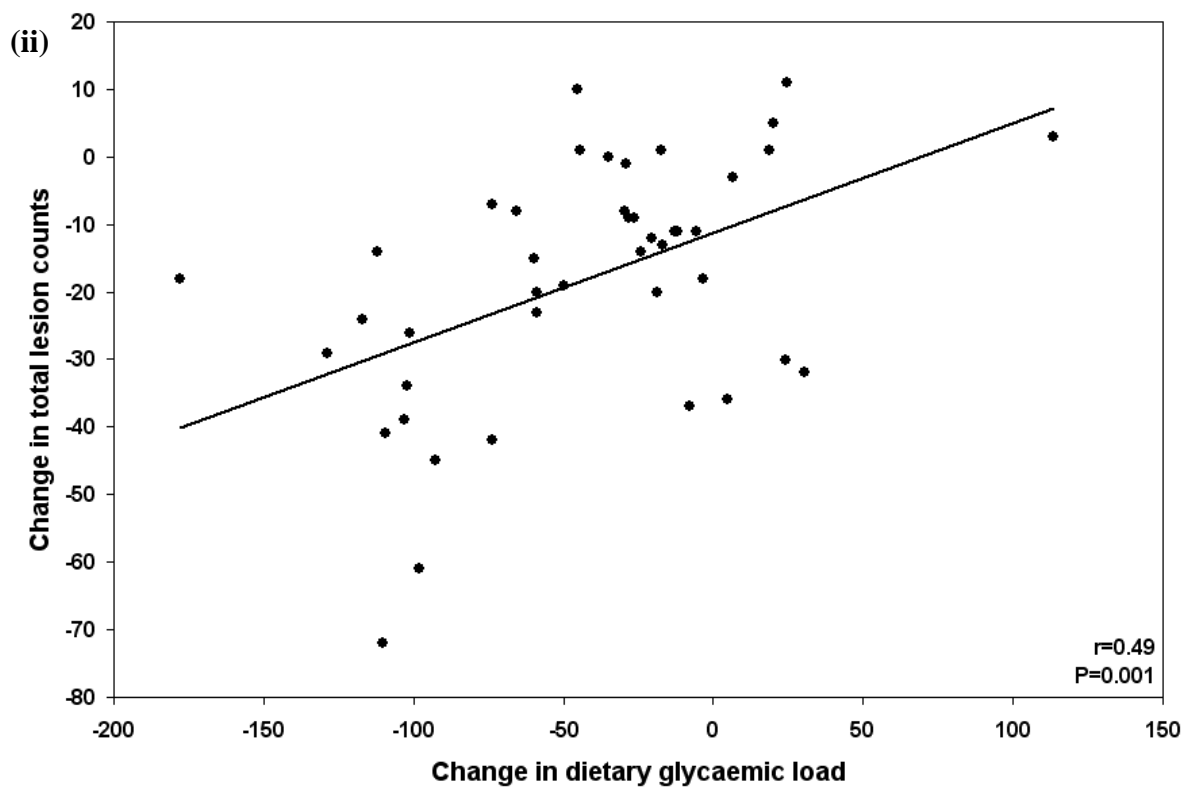
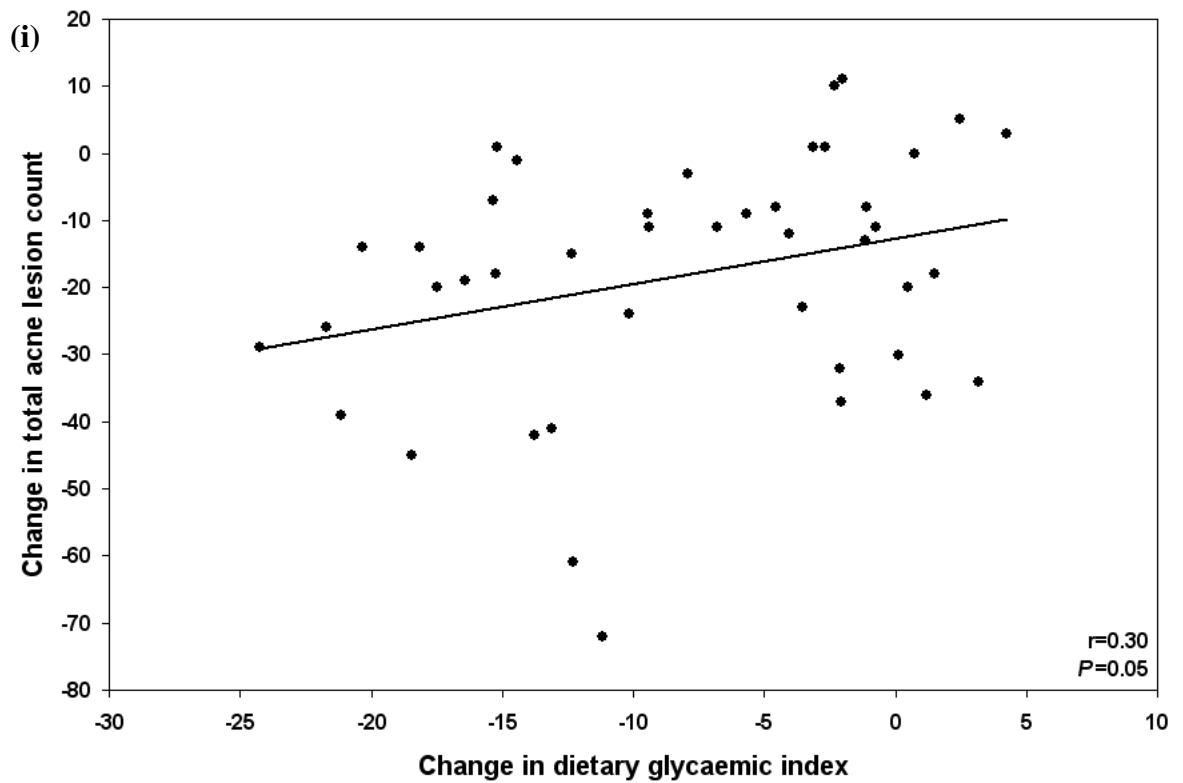
³ Mean change in HOMA-IR is non-parametrically distributed. ANCOVA was performed on log transformed data.

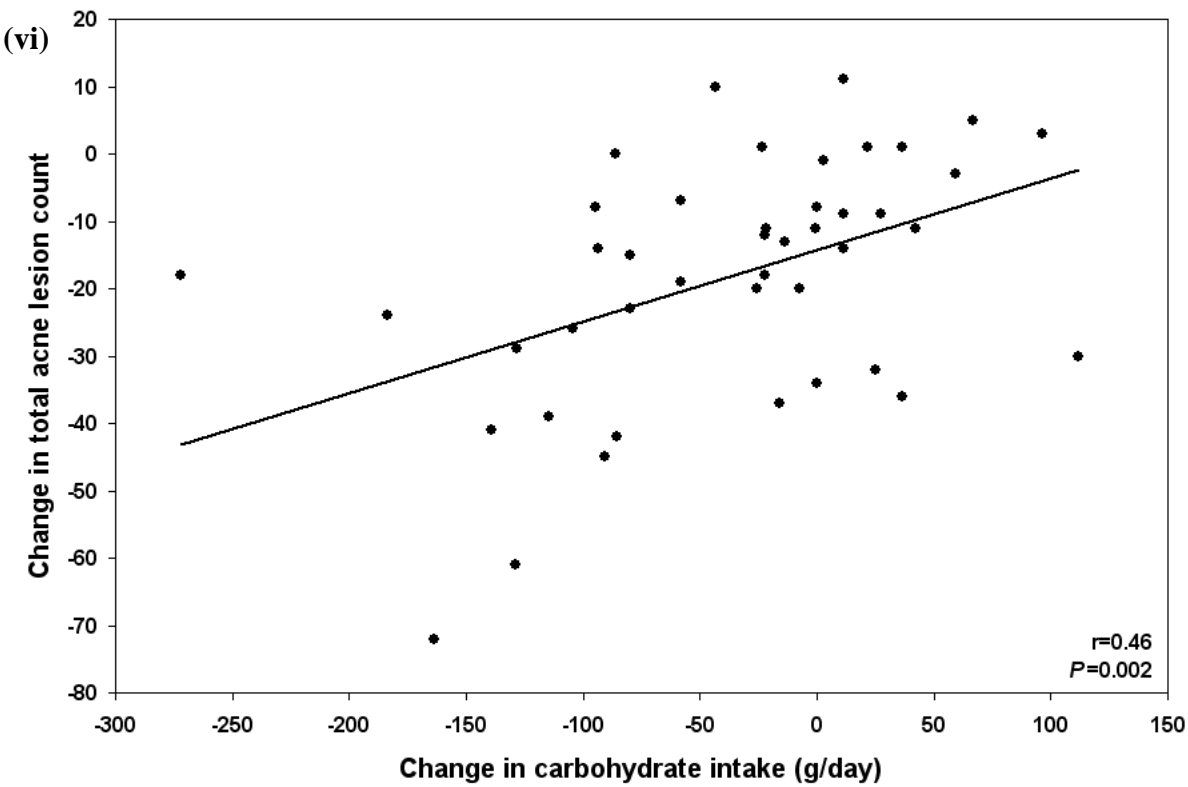
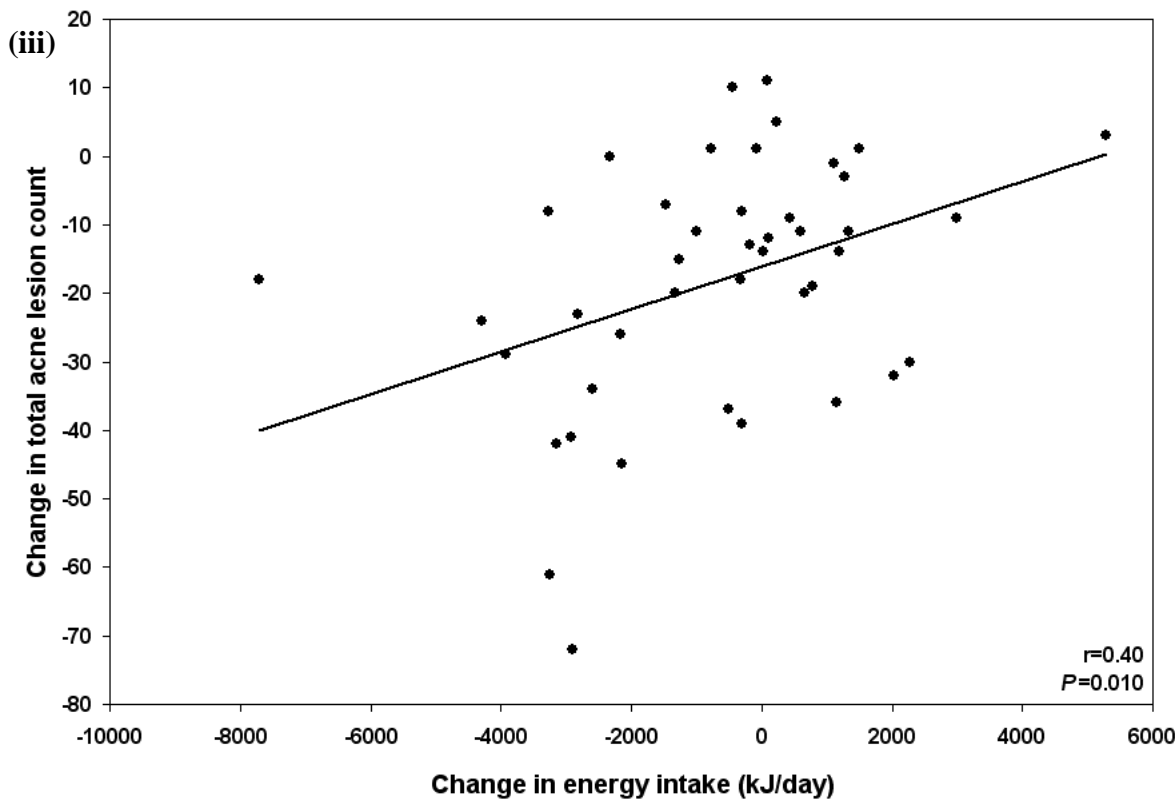


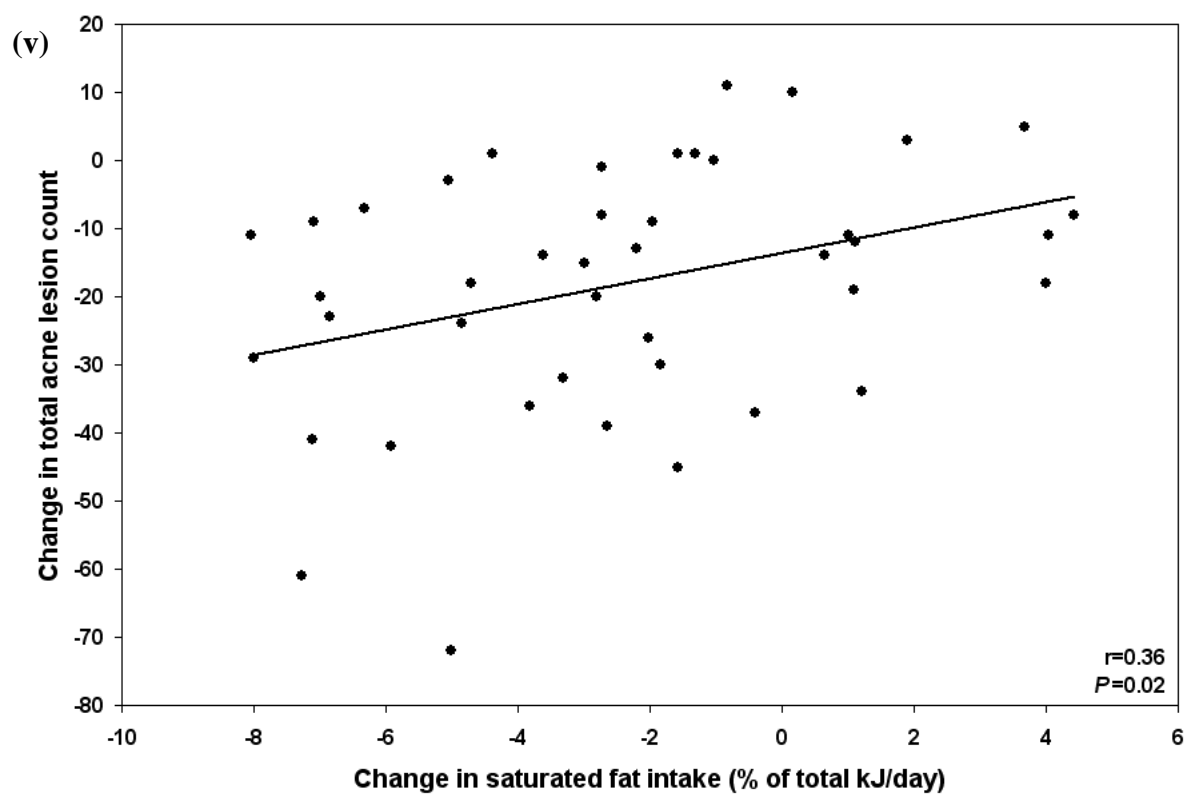
Figures 3.3: Photographs of acne improvement in the low glycaemic load group. (i) and (ii): Subject A at baseline and 12 weeks respectively; (iii) and (iv): Subject B at baseline and 12 weeks respectively; and (v) and (vi): Subject C at baseline and 12 weeks respectively

Figure 3.4: Relationships between acne improvement and changes in dietary glycaemic index, dietary glycaemic load, energy intake, carbohydrate intake, and percentage saturated fat.

Bivariate analysis was performed with a 2-tailed Pearson's correlation ($n=43$)







3.6 DISCUSSION

This study represents the first randomised controlled intervention to examine the influence of dietary glycaemic load on the clinical assessment of acne. After adjusting for differences in acne severity at baseline, we found that the LGL group showed a greater overall reduction in total and inflammatory lesion counts than did the control group. Analysis of the primary endpoint data also showed that the differences between groups remained significant after using an intention-to-treat model. However, we found no significant effect of time on acne possibly due to the fact that acne is a dynamic condition in which both spontaneous improvements and flares may occur over time.

Few well-controlled dietary studies have examined the effect of diet on acne. Fulton *et al* (1969), in a crossover single-blind study, found no effect of chocolate on acne when compared to a placebo bar. However, a later examination of the ingredients in the placebo bar indicated that the fatty acid composition and sugar contents were virtually identical to that found in the chocolate (Mackie and Mackie, 1974). Anderson (1971) examined the effect of the daily consumption of chocolate, milk or nuts and found no effect on acne. However, this study has also been criticised for its small sample size, the short follow-up and lack of controls (Margin *et al.*, 2005). Chiu *et al* (2003) showed, in university students, an association between worsening diet quality and exacerbation of acne during a preexamination period. However, stress was found to be the main contributing factor, and diet was assessed using a non-quantitative, self-assessed measure of diet quality. Recently, a retrospective evaluation of dietary intake revealed a positive association between milk intake and physician-diagnosed severe acne (Adebamowo *et al.*, 2005). However, the authors admit that this association may have been affected by the imprecision inherent in the measurement of dietary intakes via distant recall.

High glycaemic load diets have recently been implicated in acne aetiology because of their ability to increase insulin demand and other factors associated with insulin resistance (eg. hyperphagia, elevated nonesterified fatty acids and obesity) (Cordain *et al.*, 2003). Clinical and experimental evidence suggests that insulin may increase androgen production and affect, through its influence upon steroidogenic enzymes (Kristiansen *et al.*, 1997), gonadotrophin releasing hormone secretion (Willis *et*

al., 1996) and sex hormone binding globulin production (Plymate *et al.*, 1995; Haffner, 1996; Goodman-Gruen and Barret-Connor, 1997). Additionally, insulin has been shown to decrease a binding protein for IGF-I, which may facilitate the effect of IGF-I on cell proliferation (Powell *et al.*, 1991). Overall, these events may influence one or more of the four underlying causes of acne: 1) increased proliferation of basal keratinocytes within the pilosebaceous duct, 2) abnormal desquamation of follicular corneocytes, 3) androgen-mediated increases in sebum production, and 4) colonisation and inflammation of the comedo by *Propionibacterium acnes* (Cordain *et al.*, 2003).

The role of insulin in acne development is also supported by the high prevalence of acne in women with polycystic ovary syndrome (PCOS), a condition associated with insulin resistance, hyperinsulinaemia and hyperandrogenism (Franks, 2003). Insulin resistance is believed to be the underlying disturbance in PCOS, as it generally precedes and gives rise to the cluster of endocrine abnormalities that characterise PCOS (elevated androgen and IGF-I concentrations and low sex hormone binding globulin) (Dunaif *et al.*, 1989). Treatments for PCOS now include oral hypoglycaemic agents which improve insulin sensitivity, restore fertility and alleviate acne (Bourne and Jacobs, 1956).

Our study also suggests that changes in acne may be closely related to changes in insulin sensitivity, as we observed a positive effect of the LGL diet on insulin sensitivity when compared to the control diet. However, the improvement in insulin sensitivity may be attributable not only to the reduction in glycaemic load (Frost *et al.*, 1996), but also to the reduction in body mass. The participants in the LGL group lost weight despite receiving dietary advice to maintain their baseline energy intake. This may be due to the dual effect of added protein and low-GI foods, as both influence hunger and satiety. Feeding studies have shown that low-GI foods increase satiety, delay hunger and decrease food intake when compared with high-GI foods (Ludwig, 2000; Ball *et al.*, 2003). Similar effects upon satiety have been reported for high-protein meals compared to isocaloric high-carbohydrate or high-fat meals (Poppitt *et al.*, 1998). Therefore, the combined effect of low-GI foods and added protein may synergistically reduce *ad libitum* food intake, which made it difficult for our participants to maintain the energy density of their baseline diets. This observation is supported by previous studies that

showed *ad libitum* LGL diets to reduce energy intake without the need for an externally imposed energy restriction (Spieth *et al.*, 2000; Ebbeling *et al.*, 2003).

Because the participants on the LGL diet lost weight, we cannot preclude a role of the change in BMI in the overall treatment effect. When we statistically adjusted the data for changes in BMI, the effect of the LGL diet on total lesion counts and HOMA-IR was lost. This suggests that the therapeutic effect may be a factor of the weight loss, or simply that weight loss is another manifestation of a LGL diet. Apart from women with PCOS, there is little evidence to suggest an association between acne severity and body weight. Aizawa and Niimura (1996) demonstrated mild peripheral insulin resistance in female acne sufferers that was not associated with obesity or menstrual irregularities. In contrast, Bourne and Jacobs (1956) demonstrated that adult men with acne were significantly heavier (5.6kg) than men without acne. However, the authors demonstrated that this association was dependent on age, as weight was not associated with acne in adolescents aged 15-19yr. Our data also showed a significant correlation between acne lesion counts and BMI in males aged 18-25yr, but this was not true for the subjects aged <18yr (data not shown). The reason for this observation is unknown, but it is possible that the transient decline in insulin sensitivity that occurs with the progression through puberty (Bloch *et al.*, 1987; Caprio *et al.*, 1989) may influence acne in the younger population.

A few limitations of the study should be addressed. First, it is possible that the topical application of the mild skin cleanser may have contributed to the acne improvement through effects on the epidermal barrier function (Draelos, 2006). Because acne improved in the control group without any significant changes to their diet, a possible direct effect of the cleanser should be considered. Second, because of the nature of LGL dietary intervention, we cannot solely attribute the treatment effects to changes in glycaemic load as other dietary factors (eg. zinc and vitamin A intake) may mediate or confound the relationship between diet and acne improvement. Last, this study relied upon self-reporting of dietary intakes. Underreporting of the quantity of food eaten is a known source of measurement error when assessing adolescent diets (Livingstone and Robson, 2000).

To our knowledge, this is the first study to demonstrate a therapeutic effect of dietary intervention on acne. After 12 weeks, a LGL diet was shown to significantly reduce acne lesion counts and improve insulin sensitivity when compared to a high-glycaemic-load diet. Although we could not isolate the effect of the LGL diet from that of weight loss, these findings are consistent with earlier suggestions of the association between hyperinsulinaemia and acne. These observations will need to be substantiated and the underlying mechanisms determined in larger-scale studies.

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CHAPTER FOUR

The effect of a high protein, low glycaemic load diet versus a conventional, high glycaemic load diet on biochemical parameters associated with *acne vulgaris*

A randomised, investigator-masked, controlled trial

Robyn N. Smith¹, Neil J. Mann¹, Anna Braue², Henna Mäkaläinen⁴, George A. Varigos^{2,3}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Department of Dermatology, Royal Melbourne Hospital, Parkville, Australia

³Department of Dermatology, Royal Children's Hospital, Parkville, Australia

⁴Department of Biochemistry and Food Chemistry, Turku University, Finland

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4.1 ABSTRACT

Background: No previous study has sought to examine the influence of dietary composition on *acne vulgaris*.

Objective: We sought to compare the effect of an experimental low glycaemic load diet with a conventional high glycaemic load diet on clinical and endocrine aspects of *acne vulgaris*.

Methods: A total of 43 male patients with acne completed a 12 week, parallel, dietary intervention study with investigator-masked dermatology assessments. Primary outcomes measures were changes in lesion counts, sex hormone binding globulin, free androgen index, insulin-like growth factor-I and insulin-like growth factor binding proteins.

Results: At 12 weeks, total lesion counts had decreased more in the experimental group [-21.9 (95% confidence interval, -26.8 to -19.0)] compared to the control group [-13.8 (-19.1 to -8.5), $P=0.01$]. The experimental diet also reduced weight ($P=0.001$), reduced the free androgen index ($P=0.04$) and increased IGF binding protein-1 ($P=0.001$) when compared with a high glycaemic load diet.

Limitations: We could not preclude the role of weight loss in the overall treatment effect.

Conclusion: This suggests nutrition-related lifestyle factors play a role in acne pathogenesis. However, these preliminary findings should be confirmed by similar studies.

4.2 INTRODUCTION

The pathogenesis of acne is complex, with strong evidence supporting the involvement of sebaceous hyperplasia, follicular hyperkeratinisation, bacterial and yeast intrafollicular colonisation and inflammation (Deplewski and Rosenfield, 2000). Although androgens play an essential role in the development of acne, few studies have demonstrated a direct correlation between acne severity and plasma androgen levels (Shaw, 2002). Variations in the clinical response to androgens suggests that the endocrine control of acne is complex (Levell *et al.*, 1989). Some studies suggest that acne severity correlates better with sex hormone binding globulin (SHBG) than circulating testosterone levels (Odland *et al.*, 1982; Lawrence, 1986; Slayden *et al.*, 2001). Elevated dehydroepiandrosterone sulphate (DHEA-S), the major adrenal androgen, has also been shown to correlate with acne severity in adolescent girls and with lesion counts in adults (Lucky *et al.*, 1983; Cappel *et al.*, 2005). Other biological factors, such as insulin and insulin-like growth factor (IGF)-I, may also augment sebum production, one of the four proximate causes of acne. Clinically, IGF-I has been shown to correlate with acne lesion counts in adult women and significantly higher IGF-I levels have been described in women with acne compared with controls (Aizawa and Niimura, 1995; Deplewski and Rosenfield, 1999; Cappel *et al.*, 2005).

Acne is also a common feature of women with polycystic ovary syndrome (PCOS), a condition characterised by hyperandrogenism and hyperinsulinaemia (Franks, 2003). Clinical and experimental evidence suggests that insulin resistance and its compensatory hyperinsulinaemia are the underlying disturbance in PCOS, as insulin resistance generally precedes and gives rise to hyperandrogenism (Dunaif *et al.*, 1989). Insulin has been shown to stimulate ovarian androgen production through effects on steroidogenic enzymes and by amplifying gonadotrophin releasing hormone secretion (Willis *et al.*, 1996). Insulin and IGF-I stimulate adrenal androgen synthesis (Kristiansen *et al.*, 1997) and inhibit hepatic SHBG production (Singh *et al.*, 1990), allowing for an increase in androgen bioavailability. Furthermore, insulin has been shown to decrease IGF-binding protein 1 (IGFBP-1), allowing free IGF-I concentrations to act on target tissues (Powell *et al.*, 1991). Treatments aimed at reducing insulin secretion and/or increasing insulin sensitivity, such as metformin or acarbose, have been shown to

improve clinical symptoms of acne in patients with PCOS (Kolodziejczyk *et al.*, 2000; Ciotta *et al.*, 2001; Kazerooni and Dehghan-Kooshkghazi, 2003).

Expression of acne during adolescence may also be affected by endocrine changes, which are closely related to changes in insulin sensitivity. During normal puberty and adolescence, there is a transient decline in insulin sensitivity (Caprio *et al.*, 1989; Smith *et al.*, 1989), which is accompanied by a reciprocal decrease in levels of SHBG and IGFBP-1 (Holly *et al.*, 1989). According to cross-sectional observations, acne begins about the same time as the gradual increase in plasma insulin (Smith *et al.*, 1989), the preadolescent rise in body mass index (BMI) (Rolland-Cachera, 1993), and the increase in IGF-I concentrations (Caprio *et al.*, 1989; Smith *et al.*, 1989). Acne incidence more closely corresponds to the changing course of insulin and IGF-I levels than to changes in plasma androgens. This is because insulin and IGF-I levels peak during late puberty and gradually decline until the third decade (Smith *et al.*, 1989). Acne generally resolves by this time despite circulating androgens remaining unchanged.

Hyperinsulinaemia may provide an important link between nutrition-related lifestyle factors and the incidence of acne. Accumulating evidence suggests that low glycaemic load (LGL) diets may play a dual role in the prevention of hyperinsulinaemia by lowering the postprandial insulin demand and improving insulin sensitivity (Jenkins *et al.*, 2002; Willett *et al.*, 2002; Carnethon *et al.*, 2004; McKeown *et al.*, 2004). Dietary glycaemic load may be interpreted as a measure of the blood glucose and insulin-raising potential, as it represents the rate of carbohydrate absorption (indicated by the glycaemic index [GI]) and the quantity of carbohydrate consumed (Brand-Miller *et al.*, 2003). It has recently been postulated that high intakes of refined, high-GI carbohydrates may be a significant contributor to the high incidence of acne in Western countries (Cordain *et al.*, 2002). However, the impact of such a dietary change on acne and hormone levels has not been previously investigated. Therefore, the aim of the present study was to investigate the effect of a LGL diet, compared with a typical high-glycaemic-load diet, on acne severity and metabolic and endocrine variables associated with insulin resistance.

4.3 SUBJECTS AND METHODS

4.3.1 *Study population*

Male acne sufferers were recruited using flyers posted around RMIT University (Melbourne, Australia) and newspaper advertisements. Informed consent was obtained from participants and guardians (if aged <18 yr) and the study was conducted at RMIT University with the approval of the RMIT Human Ethics committee. This study included only male participants, aged 15-25 with mild-moderate facial acne. Eligible participants were required to have acne for more than 6 months and a severity grade of >0.25 but <2.0 as defined by the Leeds acne grading technique (Burke and Cunliffe, 1984). Volunteers were excluded if they were taking medications known to affect acne or glucose metabolism. A wash-out period of 6-months was required for oral retinoids or 2-months for oral antibiotics or topical agents.

4.3.2 *Study Design*

It was calculated that 20 subjects per group would provide 80% power (at the two-sided 5% level) to detect a difference of 20% in acne lesion counts, 2.7 μ U/L in fasting insulin, 5.5nmol/L in SHBG and 8.3ng/mL in IGFBP-1. To compensate for expected subject withdrawal, 54 subjects were enrolled.

Eligible participants were recruited between June 2003 and June 2004. Approximately 2-3 weeks following recruitment, participants attended their baseline appointment and were randomly assigned (1:1) to either the low glycaemic load (LGL) or control group (see **Figure 4.1**). Randomisation was carried out by computer generated random numbers and allocation to groups was performed by a third party.

This study was designed as a parallel dietary intervention study with investigator-masked dermatology assessments. Topical therapy, in the form of a non-comedogenic cleanser, was standardised for both groups and facial acne was scored at monthly visits (weeks 0, 4, 8, and 12) at the academic research clinic. On all visits, height, weight, percentage body fat and hip and waist circumference were measured. All subjects were weighed in light clothes and body mass index (BMI) was calculated as:

weight (kg)/height (m)². Percentage body fat was measured using a bio-electrical impedance analyser (TBF-521, Tanita, Illinois, USA). At baseline and 12 weeks, a venous blood sample was taken after an overnight fast.

4.3.3 Dietary intervention

Participants were informed that the study was comparing the carbohydrate to protein ratio in the diet and were not informed of the study's hypothesis. The LGL diet was low in glycaemic load, achieved through modifications to the amount and type of carbohydrate. The LGL group were educated on how to substitute high-GI foods with foods higher in protein (eg. lean meat, poultry or fish) and lower in GI (eg. whole grain bread, pasta, fruits). Some staple foods were supplied and participants were urged to consume these or similar foods on a daily basis. Each participant's dietary directions were isocalorically matched with their baseline diet as determined from 7-day weighed/measured food records. The recommended LGL diet consisted of 25% energy from protein, 45% from low-GI carbohydrates and 30% energy from fats. In contrast, the control group received carbohydrate-dense staples and were instructed to eat these or similar foods daily. The foods provided had moderate to high-GI values and were typical of their normal diet as evidenced from 7-day weighed/measured food records. The control group were not informed about the GI, but were urged to include carbohydrates as a regular part of their diet. All participants received initial education on how to use foods scales and keep foods records. During the study period, nutrient intakes were calculated from 3-day weighed/measured food records each month using Australia specific dietary analysis software (Foodworks, Xyris Software, Highgate Hill, Australia). Dietary compliance was monitored by regular telephone interviews, assessments of GL /day and 24-hr urine samples (wks 0 and 12) for an assessment of urea excretion relative to urinary creatinine.

4.3.4 Standardised topical cleanser

All subjects were provided with a topical cleanser (Cetaphil® gentle skin cleanser, Galderma, Forrester Hill, Australia) and were advised to use it in place of their normal wash, soap or cleanser. The cleanser provided contained no active agents for acne and its formulation is identical to that which is currently

available overseas. Subjects began using the topical cleanser 2 weeks prior to baseline and were asked to maintain a standard level of usage during the trial. Compliance was determined from self-report at each visit.

4.3.5 Dermatology assessment

Scaling of the acne was performed by a dermatology registrar who was masked to the group assignment of the participants. The registrar assessed facial acne occurrence and severity using a modified Burke and Cunliffe (Leeds) lesion count technique (Burke and Cunliffe, 1984). To ensure all acne lesions were counted, located and graded by size and severity, lesions were mapped by placing a transparent plastic film with a laser-printed grid gently against the skin. Facial anatomical landmarks, such as the ear, chin and tip of nose, were used to ensure consistency between assessments. Each side of the face was assessed separately. Where necessary, the registrar palpated the skin to determine the lesion type. All assessments were performed under fluorescent background lighting and with a halogen lamp, which could be easily moved to illuminate both sides of the subject's face. To maintain reproducibility of the above method, one physician performed all the dermatology assessments. A small group of volunteers (n=4) was counted one-week apart to evaluate the reproducibility by the same physician [9.5% coefficient of variation (CV)].

4.3.6 Biochemical measurements

Code labelled samples were stored at -80°C for analysis post-study by an independent laboratory. Baseline and 12 week samples for each participant were included in the same assay run to avoid inter-assay variability. Serum insulin was measured using a commercially available microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan; intra-assay CV: 4.0%). Capillary blood glucose was measured using a Glucose 201+ analyser (HemoCue, Sweden; intra-assay CV = 1.6%). The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a surrogate measure of insulin sensitivity, calculated as $\text{fasting glucose (mmol/L)} \times \text{insulin } (\mu\text{U/mL}) \div 22.5$ (Matthews *et al.*, 1985). SHBG concentrations were assayed with a commercially available radioimmunoassay (Orion Diagnostica, Espoo, Finland; intra-assay CV = 2.5%). Total testosterone was measured using solid-

phase radioimmunoassay (Diagnostic Products, Los Angeles, USA; intra-assay CV: 2.7%). The Free Androgen Index (FAI) was calculated as testosterone concentration (nmol/L) \times 100 \div SHBG concentration (nmol/L). IGF-I (intra-assay CV: 2.9 %) and DHEA-S (intra-assay CV: 8.1%) were measured using semi-automated Immulite technology (Diagnostic Products, Los Angeles, USA). IGFBP-1 and IGFBP-3 were assayed with a non-commercial radioimmunoassay as previously described (Baxter and Martin, 1986; Baxter *et al.*, 1987). An automated Olympus analyser (Melville, USA) was used to measure total cholesterol, high density lipoprotein cholesterol and triglyceride levels (intra-assay CV were 1-2% for all tests). Low-density lipoprotein cholesterol was calculated by the Friedewald formula (Friedewald *et al.*, 1972).

4.3.7 Statistical analysis

All statistical analyses were performed for the per protocol population using statistical software (SPSS 11.0 for Windows, SPSS Inc, Chicago, Ill) and significance was set at *P* less than 0.05. Baseline variables were analysed for between group significance using Mann-Whitney *U* test or one-way ANOVA, depending on whether the data was normally distributed. General linear regression models were used to test for overall treatment differences, with adjustments made for potential cofounders, including age, ethnicity and baseline data. Secondary analyses of acne end points and hormone variables were performed adjusting for changes in BMI. All covariates were individually entered into the models to determine whether there was an interaction between the covariate and the treatment. Bivariate linear regression analysis was also conducted, pooling data from both groups, to explore relationships between endocrine variables and the change in lesion counts.

4.4 RESULTS

4.4.1 Subjects

In all, forty-three subjects completed the study per protocol (**Figure 4.1**). Seven participants did not complete the study (five in control and two in LGL groups) and four were removed from data set (two were non-compliant; two began medications known to affect acne, an exclusion criterion). Baseline characteristics are shown in **Table 4.1**.

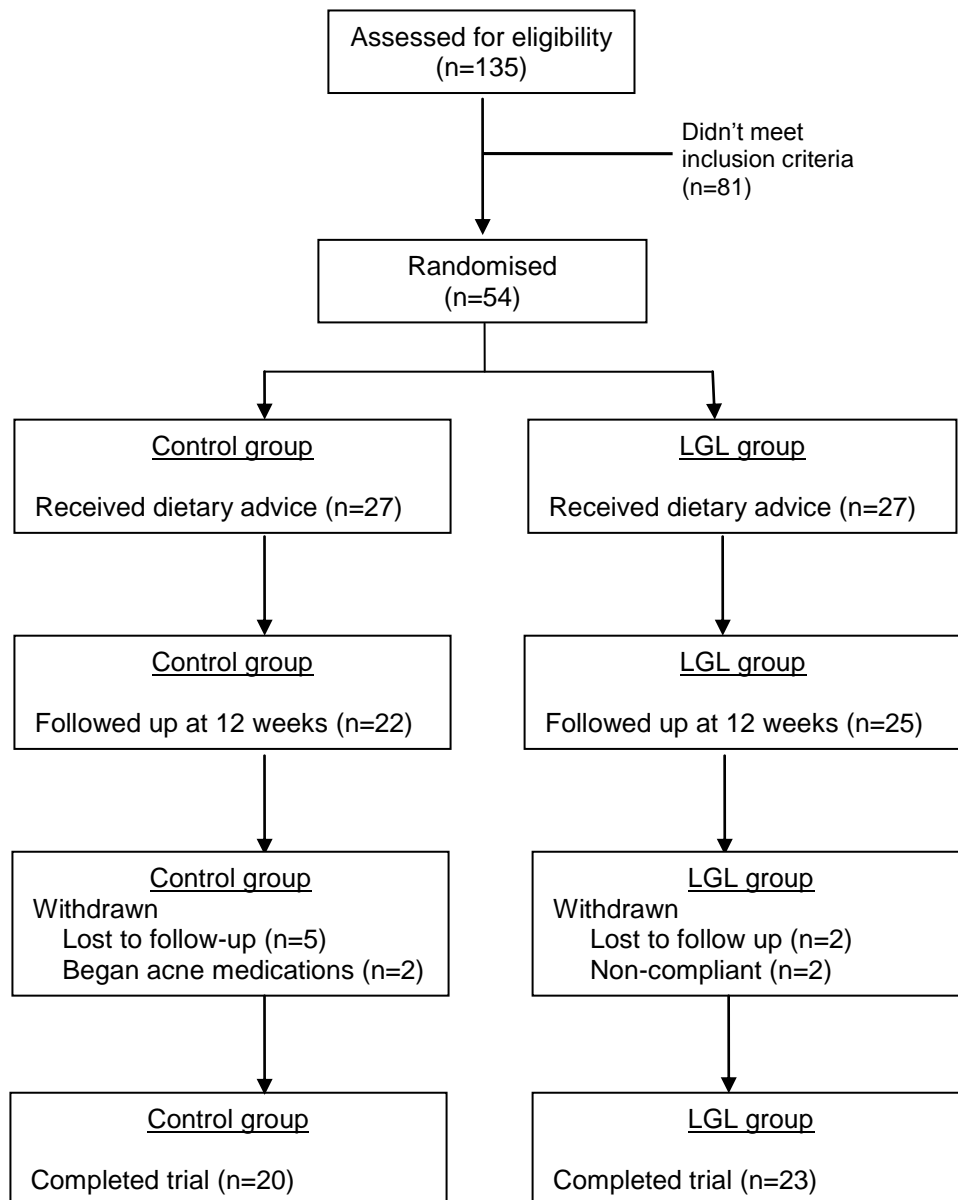


Figure 4.1: Recruitment to completion of participants in trial.

Table 4.1: Baseline characteristics of the participants by dietary group for the per protocol population

| Variable | LGL (n=23) | Control (n=20) | <i>P</i> |
|--------------------------------------|--------------|----------------|----------|
| Age (yr) | 18.2 ± 0.5 | 18.5 ± 0.5 | 0.76 |
| Body Mass Index (kg/m ²) | 22.9 ± 0.6 | 22.5 ± 0.7 | 0.34 |
| Weight (kg) | 73.5 ± 2.5 | 73.3 ± 3.3 | 0.90 |
| Waist circumference (cm) | 79.2 ± 1.7 | 79.0 ± 2.2 | 0.81 |
| Total count average | 40.6 ± 5.0 | 34.9 ± 4.3 | 0.40 |
| Inflammatory count average | 31.9 ± 3.9 | 28.4 ± 3.6 | 0.72 |
| Non-inflammatory count average | 8.8 ± 2.2 | 6.5 ± 1.6 | 0.62 |
| Fasting glucose (mmol/L) | 4.76 ± 0.07 | 4.47 ± 0.09 | 0.02 |
| Fasting insulin (mU/L) | 7.37 ± 0.66 | 7.35 ± 0.74 | 0.99 |
| Testosterone (nmol/L) | 20.97 ± 1.19 | 20.36 ± 1.48 | 0.75 |
| SHBG (nmol/L) | 25.39 ± 1.27 | 24.75 ± 1.92 | 0.78 |

4.4.2 Diet composition

Table 4.2 lists the diet composition of the LGL and control groups before randomisation and during the trial. No significant group differences were observed in any of the dietary variables at baseline. During the trial period, dietary glycaemic load was significantly lower in the LGL group compared with the control group and this was achieved by a reduction in carbohydrate intake and by means of low-GI foods (as indicated by a reduction in the calculated dietary GI; see **Table 4.2**). Protein intake increased in the LGL compared with the control group ($P<0.001$), indicating that some carbohydrates were replaced with foods higher in protein. This was substantiated by a 15.4% increase in urinary urea/creatinine ratio at 12 weeks for the LGL group compared with a 12.3% decrease for the control group ($P=0.009$), indicating good dietary compliance. Although the LGL group received isocaloric dietary advice, energy intake decreased relative to their baseline diet ($P=0.02$).

4.4.3 Compliance with topical non-acne cleanser

There was no discontinuation of the topical non-acne therapy amongst the study completers.

Table 4.2: Dietary composition of the LGL and control diets before randomisation and during the trial period.

| Nutrient | Before randomisation | Trial period | | |
|--------------------------------------|----------------------|-------------------|----------------|----------|
| | (n=43) | LGL (n=23) | Control (n=20) | <i>P</i> |
| Energy (kJ/day) | 10585 | 9320 ^a | 10620 | 0.06 |
| Dietary glycaemic index [†] | 57 | 43 ^a | 54 | <0.001 |
| Dietary glycaemic load [‡] | 178 | 101 ^a | 174 | <0.001 |
| Carbohydrate, % total kJ | 49 | 44 ^a | 50 | <0.001 |
| Protein, % total kJ | 17 | 23 ^a | 17 | <0.001 |
| Total fat, % total kJ | 32 | 31 | 32 | 0.74 |
| Fat subgroups, % total kJ | | | | |
| Saturated | 13.2 | 9.0 ^a | 12.6 | <0.001 |
| Polyunsaturated | 4.5 | 6.8 ^a | 4.8 | 0.001 |
| Monounsaturated | 11.7 | 12.4 | 11.9 | 0.51 |
| Cholesterol, mg/d | 296 | 309 | 304 | 0.93 |
| Dietary fibre, g/day | 25.3 | 36.9 ^a | 26.2 | <0.001 |

P value corresponds with one-way ANOVA or Mann-Whitney.

^a Significantly different from baseline ($P < 0.05$)

[†] Calculated as the weighted average glycaemic index of all carbohydrate-containing foods in the diet.

[‡] Calculated as the food's carbohydrate amount (grams) \times the respective glycaemic index value and divided by 100, then totalled for all foods each day.

4.4.4 Study outcomes

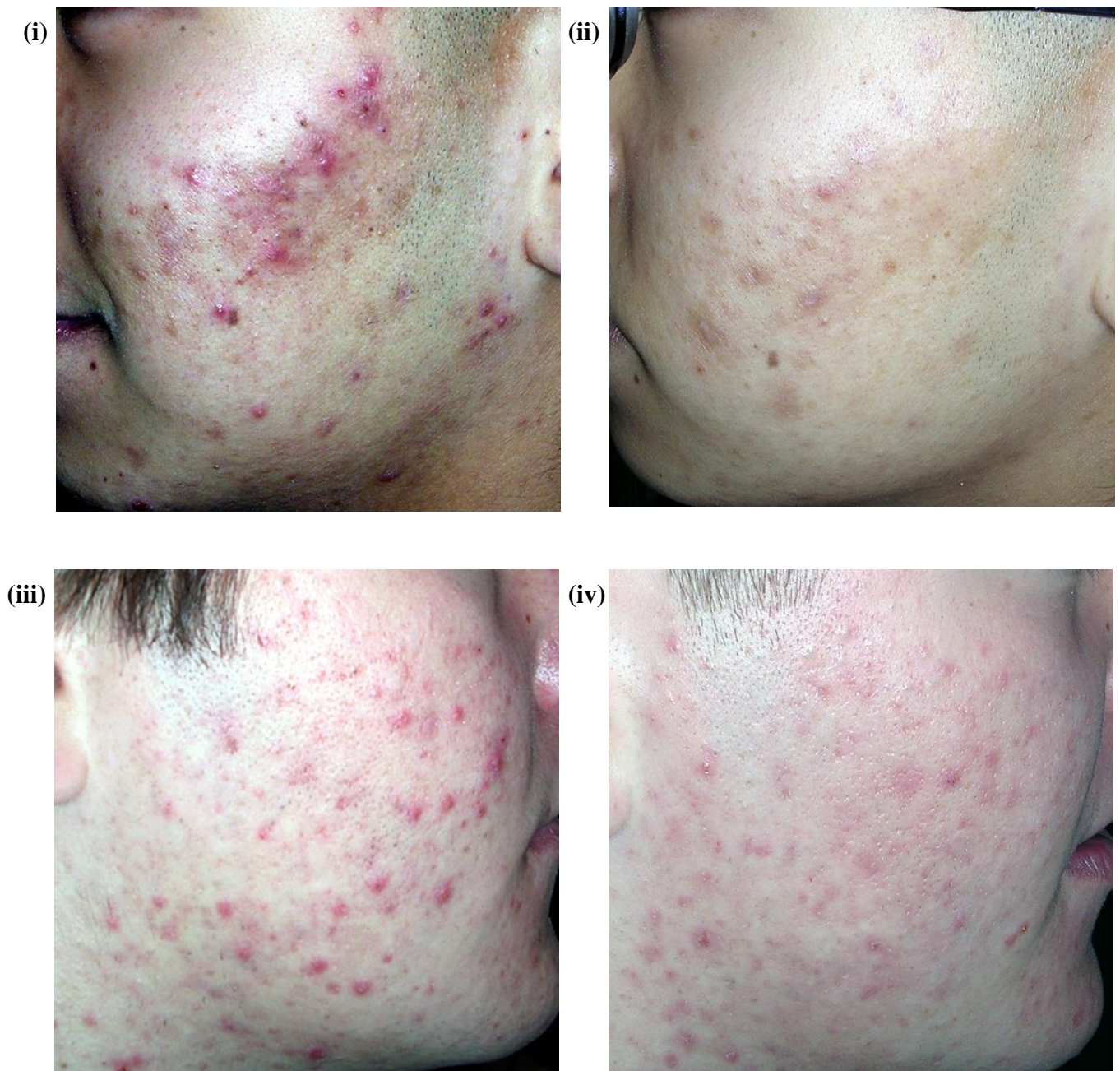
Table 4.3 shows the mean change in lesion counts and anthropometric measures at 12 weeks according to dietary group. After adjusting for age, ethnicity and baseline counts, the reduction in total lesion counts was significantly greater in the LGL group compared to the control group ($P=0.01$). Similar results were observed for the mean decrease in inflammatory counts ($P=0.02$). **Figure 4.3** shows examples of acne improvement in the LGL group. The LGL group also showed significantly greater reductions in weight ($P<0.001$), BMI ($P=0.001$), body fat percentage ($P=0.006$) and waist circumference ($P=0.04$) when compared to the control group. Statistical adjustment of the mean change in acne scores for changes in BMI altered the outcome for total lesion counts [-21.6 (95% CI, -28.9 to 14.4) vs -14.1 (-21.9 to -6.2), $P=0.07$], but not inflammatory counts [-16.0 (95% CI, -20.6 to -11.4) vs -8.5 (-13.4 to -3.5), $P=0.04$]. However, we found no significant interaction effect of dietary treatment and the change in BMI on acne end-points.

Table 4.4 shows the mean change in hormonal variables and plasma lipids at 12 weeks according to dietary group. The mean change in fasting insulin levels ($P = 0.03$) and logHOMA-IR ($P = 0.02$) was significantly different between groups with the LGL group showing a trend for improved insulin sensitivity and the control group showing a trend for increasing insulin resistance. SHBG levels decreased in the control group compared with the LGL group ($P = 0.031$). The effect of dietary treatment on FAI ($P = 0.041$) was marginally significant, with the LGL group showing a decrease in testosterone bioavailability compared to the control group. IGFBP-1 increased significantly in the LGL group relative to baseline ($P = 0.001$) and this was significantly different from the mean change in the control group. Statistical adjustment for changes in BMI was found to affect the results for HOMA-IR (-0.04 for the LGL group vs 0.08 for the control group, $P=0.08$), SHBG (-0.04 for the LGL group vs -2.35 for the control group, $P=0.13$), and FAI (-8.8 for the LGL group vs 4.6 for the control group, $P=0.07$) but not IGFBP-1 (0.14 for the LGL group vs -0.09 for the control group, $P=0.003$).

Table 4.3: Mean change in outcome variables for the per protocol population at 12 weeks according to dietary group

| Variable | Adjusted means (95% CI) [†] | | <i>P</i> |
|----------------------------|--------------------------------------|----------------------|----------|
| | LGL group (n=23) | Control group (n=20) | |
| Total lesion counts | -21.9 (-26.8, -19.0) | -13.8 (-19.1, -8.5) | 0.01 |
| Inflammatory lesion counts | -16.0 (-20.3, -11.8) | -8.4 (-13.0, -3.8) | 0.02 |
| Weight (kg) | -2.9 (-4.0, -1.7) | 0.4 (-0.8, 1.7) | <0.001 |
| BMI (kg/m ²) | -0.89 (-1.25, -0.54) | -0.02 (-0.41, 0.36) | 0.002 |
| Percentage body fat (%) | -2.2 (-3.0, -1.4) | -0.5 (-1.3, 0.4) | 0.006 |
| Waist circumference (cm) | -2.2 (-3.6, -0.9) | -0.2 (-1.6, 1.2) | 0.04 |

[†] Means are adjusted for differences in baseline values, age and ethnicity



Figures 4.2(i)-(iv): Photographs of acne improvement in the low-glycemic-load group. Patient A at baseline (i) and 12 weeks (ii). Patient B at baseline (iii) and 12 weeks (iv).

Table 4.4: Mean change in hormone concentrations and plasma lipids for the per protocol population at 12 weeks according to dietary group

| | Adjusted means [†] (95% CI) | | <i>P</i> |
|-------------------------------------|--------------------------------------|-----------------------------------|----------|
| | LGL (n=23) | Control (n=20) | |
| Fasting insulin (μU/L) [‡] | -0.90 (-2.59, 0.79) | 1.96 (0.15, 3.77) | 0.03 |
| Log(HOMA-IR) | -0.05 (-0.13, 0.03) | 0.09 (0.01, 0.18) | 0.02 |
| Testosterone (nmol/L) | -1.32 (-2.75, 0.12) | -1.20 (2.74, 0.34) | 0.91 |
| SHBG (nmol/L) | 0.27 (-1.56, 2.09) | -2.71 (-4.67, -0.74) ^a | 0.03 |
| FAI (nmol/L) | -8.73 (-17.34, -0.12) ^a | 4.51 (-4.74, 13.75) | 0.04 |
| DHEA-S (μmol/L) | -0.68 (-1.30, -0.06) ^a | -0.12 (-0.79, 0.55) | 0.22 |
| IGF-I (nmol/L) | -2.93 (-6.21, 0.35) | -2.79 (-6.31, 0.73) | 0.95 |
| Log(IGFBP-1) (ng/mL) | 0.14 (0.05, 0.22) ^a | -0.09 (-0.18, 0.03) | 0.001 |
| IGFBP-3 (mg/mL) | 0.11 (-0.10, 0.32) | 0.16 (-0.06, 0.38) | 0.75 |
| T-Chol (mmol/L) | -0.42 (-0.63, -0.21) ^a | -0.05 (-0.27, 0.17) | 0.02 |
| LDL-Chol (mmol/L) | -0.32 (-0.51, -0.13) ^a | -0.04 (-0.25, 0.16) | 0.06 |
| HDL-Chol (mmol/L) | -0.04 (-0.10, 0.10) | -0.05 (-0.11, 0.01) | 0.92 |
| Log(triglycerides) (mmol/L) | -0.06 (-0.13, 0.08) | 0.03 (-0.05, 0.10) | 0.10 |

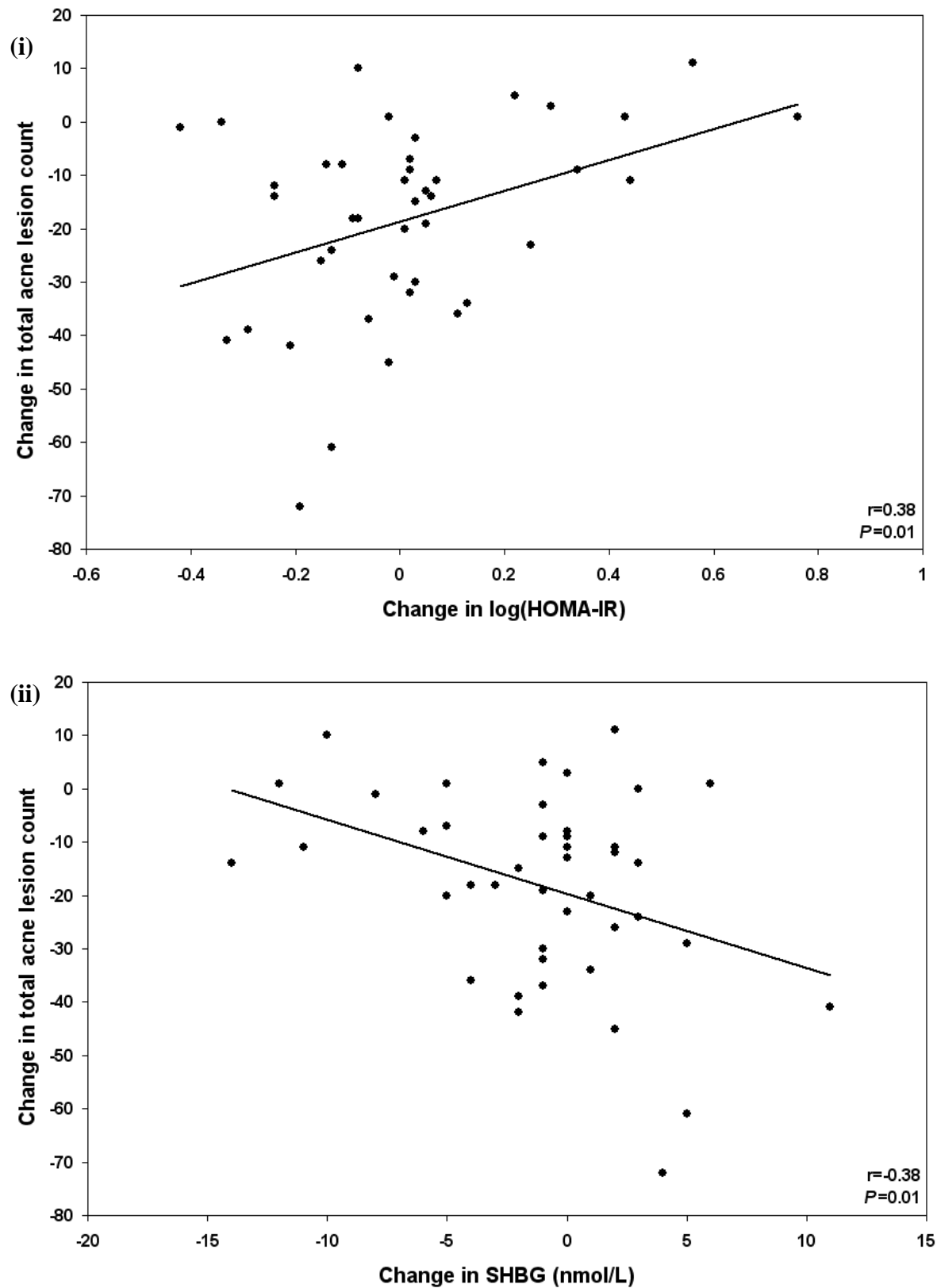
[†] Means are adjusted for differences in baseline values, age and ethnicity

[‡] n=42 due to an outlier of more than 3 SD from the mean

^a Significant difference from baseline to 12 weeks (*P*<0.05)

4.4.5 *Hormonal variables as predictors of acne improvement*

Figure 4.2 depicts the results from bivariate linear regression analysis. A positive relationship was observed between the change in total lesion counts and the change in insulin sensitivity as determined by HOMA-IR ($r = 0.38$, $P = 0.01$). A change in SHBG levels was also shown to correlate negatively with a change in lesion counts ($r = -0.38$, $P = 0.01$). In contrast, a change in FAI was not significantly associated with the change in lesion counts ($r=0.10$, $P=0.50$).



Figures 4.3: Relationships between acne improvement and hormone variables. (i) HOMA-IR and (ii) SHBG.

4.5 DISCUSSION

This pilot study investigated the independent effects of an experimental LGL diet versus a conventional high-glycaemic-load diet, combined with a standardised non-acne cleanser. Although both groups showed improvements in acne, the LGL group showed significantly greater reductions in the clinical and endocrine assessments of acne. In addition, participants in the LGL group showed reductions in weight and measures of adiposity, despite receiving dietary advice that was isocalorically matched with diets at baseline. In contrast, participants on the conventional, high-glycaemic-load diet showed no change in weight or body composition.

This study found a positive effect of a LGL diet on insulin sensitivity when compared with a conventional high-glycaemic-load diet. The improvement in insulin sensitivity may be attributable not only to the decrease in glycaemic load (Frost *et al.*, 1996), but also to the decrease in total energy intake and subsequent body weight loss (Hannah and Howard, 1994). It has been hypothesised that a decrease in insulin may mediate a reduction in underlying pathological aspects of acne (Cordain *et al.*, 2002). In accordance with this hypothesis, we observed a moderate relationship between the change in insulin sensitivity and the change in acne lesion counts. This suggests that the therapeutic effect may be a factor of the change in insulin sensitivity, or simply that improved insulin sensitivity is another manifestation of a LGL diet. An association between acne and mild peripheral insulin resistance has been previously described in healthy eumenorrheic women (Aizawa and Niimura, 1996). The authors found that acne sufferers exhibited significant hyperinsulinaemia during an oral glucose tolerance test compared to age-matched controls. This relationship was also found to be independent of obesity, as BMI was similar in both groups.

Our results suggest that the improvement in insulin sensitivity may be linked to reductions in androgenicity. We observed a reduction in testosterone bioavailability and DHEA-S concentrations in the LGL group and this may help to explain the lessening of acne severity. The observed reduction in free testosterone was probably related to the dual effect of insulin on androgen production in testicular tissues (Bebakar *et al.*, 1990) and the hepatic production of SHBG (Singh *et al.*, 1990). Plasma concentrations of DHEA-S, the major adrenal androgen, also decreased in the LGL group, possibly

owing to insulin's effect on the expression of adrenal steroidogenic enzymes (Kristiansen *et al.*, 1997). In contrast, the control group showed a decline in insulin sensitivity and SHBG concentrations. Why acne improved in the control group despite no significant change in androgen levels remains unanswered, but the possible direct effect of the topical cleansing agent should be considered (Draelos, 2006). As SHBG correlates inversely with insulin, it wasn't surprising to find that acne also correlated with the change in SHBG. Our results also corroborate previous evidence that SHBG may be a marker of acne (Odland *et al.*, 1982; Marynick *et al.*, 1983; Lawrence). However, we observed no relationship between acne severity and free testosterone levels, an association which has been demonstrated in some (Lucky *et al.*, 1983; Schiavone *et al.*, 1983), but not all studies (Levell *et al.*, 1989; Cibula *et al.*, 2000).

Normal sebaceous gland growth is also influenced by factors other than androgens, such as IGF-I (Deplewski and Rosenfield, 1999). Therefore, increased expression of IGF-I or a reduction in the level of its carrier proteins could influence acne. In the present study, IGFBP-1 levels increased significantly in the LGL group compared with the control group and we speculate that this is a compensatory adaptation to the improvement in insulin sensitivity and the reduction in basal insulin. Insulin is the principle determinant of plasma IGFBP-1 levels and low basal IGFBP-1 levels have been observed in insulin resistant individuals, possibly due to increased portal insulin overnight (Attia *et al.*, 1998). Therefore, it is possible that the LGL diet may also induce changes to the IGF system that may be clinically relevant to events involved in acne pathogenesis.

There are some limitations regarding the study design and intervention that should be addressed. Firstly, as subjects in the LGL group lost weight, we cannot preclude the change in BMI to the overall treatment effect. When we statistically adjusted the data for changes in BMI, the effect of the LGL diet on several clinical and endocrine parameters was lost. However, this does not necessarily imply that acne is influenced by weight loss per se. Weight loss trials, involving PCOS women, have consistently shown increased SHBG levels (Kiddy *et al.*, 1989; Guzick *et al.*, 1994) and decreased FAI (Kiddy *et al.*, 1989; Guzick *et al.*, 1994). However, low-fat dietary interventions in nonobese women have shown no change in SHBG levels following weight loss (Prentice *et al.*, 1990; Rose *et al.*, 1992). At

present, there is a paucity of evidence to indicate that acne is more prevalent or severe in overweight adolescents. One study revealed a relationship between weight and acne in men aged 20-40, but this was not true for adolescents aged 15-19 (Bourne and Jacobs, 1956). Although we cannot determine an aspecific effect of weight loss on acne, one could speculate that a reduction in hyperinsulinaemia, either through weight loss or dietary composition, may reduce precipitating factors involved in acne. Another limitation of the present study was the use of a fasting index to quantitatively estimate hyperinsulinaemia and insulin resistance. Although this index correlates with the euglycaemic clamp technique and has proven to be useful in large studies, its applicability to small intervention trials remains uncertain (Daly, 2003). Furthermore, this test reflects insulin action in a basal state, whereas in life much of insulin action is in the postprandial state. Therefore, it is possible that this index may provide an underestimation or overestimation of the relationship between acne and the extent of hyperinsulinaemia.

To our knowledge, this is the first study to demonstrate a therapeutic effect of dietary intervention on acne. The results of the present study open the prospect that nutrition-related lifestyle factors may affect the pathogenesis of acne. After 12 weeks, a LGL diet was shown to reduce weight, acne severity and hormonal aspects of acne (eg. testosterone bioavailability, IGFBP-1 and HOMA-IR) when compared to a conventional high-glycaemic-load diet. Although we could not determine an aspecific effect of diet from that of weight loss, the finding that insulin sensitivity correlated with acne suggests that both may be involved. Therefore, these results should be considered preliminary and larger-scale studies are needed to confirm the effect of dietary intervention on acne.

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CHAPTER FIVE

The effect of a low glycaemic load diet on *acne vulgaris* and the fatty acid composition of skin surface triglycerides

Robyn N. Smith, BAppSc (Hons)¹, Anna Braue, MBBS, MMed³, George A. Varigos MBBS, FACD, PhD^{3,4}, Neil J. Mann(Hons), BAppSc, PhD^{1,2}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Australian Technology Network, Centre for Metabolic Fitness

³Department of Dermatology, Royal Melbourne Hospital, Parkville, Australia

⁴Department of Dermatology, Royal Children's Hospital, Parkville, Australia

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5.1 ABSTRACT

Background: Dietary factors have long been implicated in acne pathogenesis. It has recently been hypothesised that low glycaemic load diets may influence sebum production based on the beneficial endocrine effects of these diets.

Objective: To determine the effect of a low glycaemic load diet on acne and the fatty acid composition of skin surface triglycerides

Methods: Thirty-one male acne patients (aged 15-25 years) completed sebum sampling tests as part of a larger 12 week, parallel design, dietary trial. The experimental treatment was a low glycaemic load diet, comprised of 25% energy from protein and 45% from low glycaemic index carbohydrates. In contrast, the control situation emphasised carbohydrate-dense foods without reference to the glycaemic index. Acne lesion counts were assessed during monthly visits. At baseline and 12 weeks, the follicular sebum outflow and composition of skin surface triglycerides were assessed using lipid absorbent tapes.

Results: At 12 weeks, subjects on the experimental diet demonstrated increases in the ratio of saturated to monounsaturated fatty acids of skin surface triglycerides when compared with controls [$-5.3 \pm 2.0\%$ (mean \pm SEM) vs $-2.7 \pm 1.7\%$, $P=0.007$]. The increase in the saturated/monounsaturated ratio correlated with improvements in acne lesion counts ($r=-0.39$, $P=0.03$). Increased follicular sebum outflow was also associated with an increase in the proportion of monounsaturated fatty acids in sebum ($r=0.49$, $P=0.006$).

Conclusion: This suggests a possible role of desaturase enzymes in sebaceous lipogenesis and the clinical manifestation of acne. However, further work is needed to clarify the underlying role of diet in sebum gland physiology.

5.2 INTRODUCTION

Dietary factors have long been implicated in the pathogenesis of acne (Rasmussen, 1977; Michaëlsson, 1981). It is well known that increased sebum production plays a fundamental role in acne (Harris *et al.*, 1983) and evidence suggests that dietary manipulation alters sebaceous gland output. Extreme caloric restriction dramatically decreases the sebum excretion rate and these changes can be reversed when a normal diet is resumed (Pochi *et al.*, 1970; Downing *et al.*, 1972). Other studies have demonstrated that increased consumption of dietary fat or carbohydrate increases sebum production (Llewellyn, 1967), and modifications to the type of carbohydrate can also alter sebum composition (MacDonald, 1964; MacDonald, 1967). Altogether, these studies suggest that the quantity and composition of foods, when changed significantly, may affect underlying mechanisms involved in sebum production.

Evidence suggests that diet may be an important source of substrate for the synthesis of sebaceous lipids (Rasmussen, 1977). Human sebum is comprised mainly of triglycerides (40-60%), wax esters (19-26%) and squalene (11-15%), with some cholesterol and cholesterol esters (Kellum, 1967; Downing *et al.*, 1969; Greene *et al.*, 1970). These lipids can be synthesised from a variety of sources (eg. glucose, acetate, and fatty acids) which serve to donate two carbon fragments (Cassidy *et al.*, 1986; Middleton *et al.*, 1988). However, some dietary lipids (especially fatty acids) can also pass unchanged from the circulation to the sebaceous cells. It is presumed that undifferentiated cells of the sebaceous gland acquire the dietary lipids whilst in the basal layer exposed to the circulation (Downing *et al.*, 1986). This notion is supported by the observation that sebum contains linoleic acid, an essential fatty acid that cannot be synthesised *in vivo* and therefore must be obtained from the diet.

The triglyceride fraction of sebum is presumably responsible for acne development (Nicolaidis, 1974; Katsuda *et al.*, 2005). Bacteria can hydrolyse sebaceous triglycerides (Shalita, 1974), liberating the fatty acids which can penetrate the follicular wall and become incorporated into the metabolism of the surrounding epidermis. The application of free fatty acids on rabbit ears or hairless mice has been shown to induce hyperkeratinisation and epidermal hyperplasia similar to that seen in comedo formation (Maeda, 1991; Katsuda *et al.*, 2005). However, the hyperkeratotic effect may not be a

feature of all fatty acids, as recent evidence suggests that only monounsaturated fatty acids (MUFAs) stimulated the morphological changes whereas the saturated fatty acids (SFAs) have little effect (Katsuda *et al.*, 2005). Human sebum is known to contain a high proportion of MUFAs, with a characteristic double bond at the $\Delta 6$ position rather than at the standard $\Delta 9$ position (Nicolaidis, 1974). The most abundant of these is sapienic acid (16:1 $\Delta 6$), which is formed by the $\Delta 6$ desaturation of palmitic acid (16:0) (Stewart *et al.*, 1986). Sapienic acid is unique to human sebum and has not been identified in other human tissues or in sebaceous gland secretions of other animals (Nicolaidis, 1974). It is presumed that this fatty acid may play a role in acne pathogenesis, however its role is not well defined (Ge *et al.*, 2003).

In the present study, we examined the influence of diet on the fatty acid composition of skin surface triglycerides. Recent evidence suggests that low glycaemic load diets may affect sebum production based on the beneficial hormonal effects of these diets (Cordain *et al.*, 2002). The glycaemic load may be interpreted as a measure of the blood glucose and insulin-raising potential of the diet, as it represents the rate of carbohydrate absorption (indicated by the glycaemic index) and the quantity of carbohydrate consumed (Brand-Miller *et al.*, 2003). Previous studies indicate that the dietary manipulation of the quality and quantity of carbohydrates can affect the composition of fatty acids in sebum (MacDonald, 1964; MacDonald, 1967). A relative excess of dietary carbohydrate (500g/day) can increase the proportion of 16:1 in sebum, however the effect on the other fatty acids of sebum is varied depending on the type of carbohydrate used (MacDonald, 1964; MacDonald, 1967). Based on these observations, one can speculate that the composition of fatty acids in sebum may vary with alterations in the dietary glycaemic load. Therefore, the objective of the present study was to determine the effect of a low glycaemic load diet on acne and the fatty acid composition of skin surface triglycerides.

5.3 MATERIALS AND METHODS

5.3.1 Study population

Male volunteers with acne were recruited for a dietary intervention study that was conducted at RMIT University (Melbourne, Australia). Informed consent was obtained from participants and guardians (if

aged <18years) and the study had the approval of the RMIT Human Ethics committee. This study included only male participants, aged 15-25 with mild-moderate facial acne. Participants were required to have had acne for longer than 6 months prior to recruitment. Volunteers were excluded if they were taking medications known to affect acne or glucose metabolism. A wash-out period of 6-months was required for oral retinoids or 2-months for oral antibiotics or topical therapy.

5.3.2 *Study design*

Eligible participants were recruited between June 2003 and June 2004. Participants were randomly assigned to either the low glycaemic load (LGL) or control group (see **Figure 5.1**) at the time of their baseline appointment. Randomisation was carried out by computer generated random numbers and allocation to groups was performed by a third party.

The experimental protocol followed a parallel, dietary intervention with monthly visits at an academic research clinic (weeks 0, 4, 8, and 12). At baseline and 12 weeks, participants also sat a 1-hour sebum sampling test. Due to scheduling interferences (eg. school, work commitments), not all of the study participants were available to sit the 1-hour sebum sampling test. **Figure 5.1** shows the proportion of participants who completed the sebum test as per protocol.

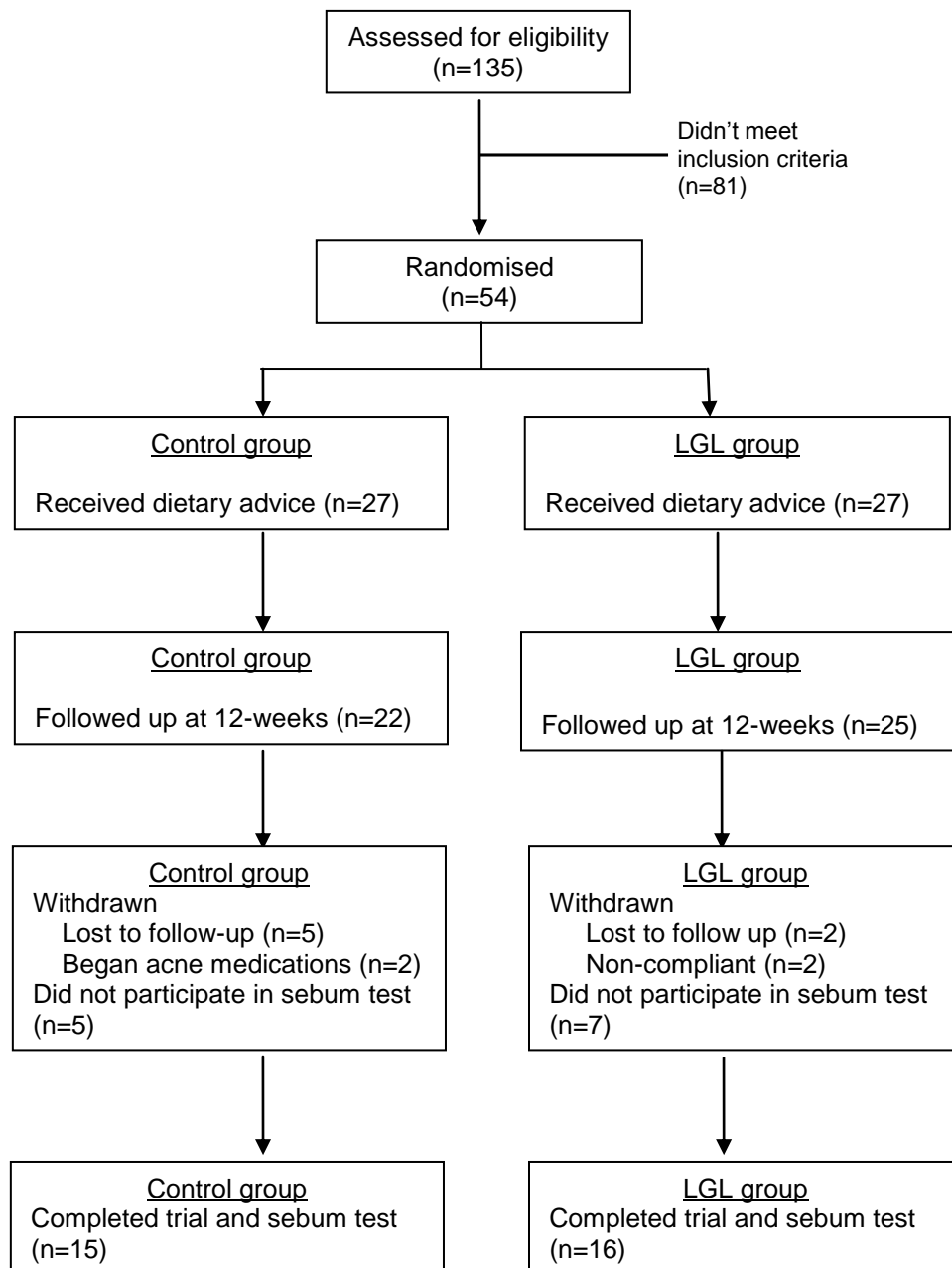


Figure 5.1: Recruitment to completion of participants in trial.

5.3.3 Dietary intervention

Participants were informed that the study's intent was to compare the dietary carbohydrate to protein ratio and were not informed of the study's true hypothesis. The LGL diet was low in glycaemic load, achieved through a reduction in carbohydrate intake and by reducing the dietary glycaemic index. The LGL group were educated on how to substitute high glycaemic index foods with foods higher in protein (eg. lean meat, poultry or fish) and lower in glycaemic index (eg. whole grain bread, pasta, fruits). Some staple foods were supplied and participants were urged to consume these or similar foods on a daily basis. Each participant's dietary directions were isocalorically matched with their baseline diet as determined from 7-day weighed/measured food records. The recommended LGL diet consisted of 25% energy from protein, 45% from low GI carbohydrates and 30% energy from fats. In contrast, the control group received carbohydrate-dense staples and were instructed to eat these or similar foods daily. The foods provided had moderate to high glycaemic index values and were typical of their normal diet as evidenced from 7-day weighed/measured food records. The control group were not informed about the glycaemic index, but were urged to include carbohydrates as a regular part of their diet. Actual dietary intakes, as assessed from weight/measured food records, have been reported elsewhere (Smith *et al.*, 2007).

5.3.4 Standardised topical cleanser

All subjects were provided with a topical cleanser (Cetaphil[®] gentle skin cleanser, Galderma, Forrests Hill, Australia) and were advised to use it in place of their normal wash, soap or cleanser. The cleanser provided was non-comedogenic and contained no active agents for acne. Subjects began using the topical cleanser 2 weeks prior to baseline and were asked to maintain a standard level of usage during the trial.

5.3.5 Dermatology assessment

Scaling of the acne was performed by a dermatology registrar who was blinded to the group assignment of the participants. The registrar assessed facial acne occurrence and severity only, using a modified Cunliffe-Leeds lesion count technique (Burke and Cunliffe, 1984).

5.3.6 *Sebutape sample collection method*

Skin surface lipids were collected using Sebutape[®] test strips (CuDerm Corporation, Dallas, USA) at baseline and 12 weeks. Sebutape[®] is a lipid absorbent strip that has been shown to be a reproducible and non-invasive method for estimating the output of sebum from active follicles (Nordstrom *et al.*, 1986).

Prior to the application of the Sebutape[®] adhesive strips, residual surface lipid was removed from the forehead using an isopropyl alcohol swab. For sampling, two lipid absorbing strips were applied to the forehead for a 1 hour collection, using disposable gloves and alcohol-rinsed forceps to prevent lipid-contamination. The forehead was chosen as the site of collection as the contribution of epidermal lipids to the total composition of skin surface lipids has been shown to be minimal (less than 10 $\mu\text{g}/\text{cm}^2$ compared with average recoveries of 150 – 300 $\mu\text{g}/\text{cm}^2$ for sebum) (Greene *et al.*, 1970). During the sampling time, the room temperature was kept between 18-21°C.

Following collection, one test strip was applied to storage card (supplied by CuDerm) for photometric analysis and the second strip was placed in a chloroform-methanol rinsed, teflon-capped glass vial. The glass vial was flushed with nitrogen and then frozen at -80°C for later compositional analysis.

5.3.7 *Photometric assessment of the follicular sebum outflow*

High resolution photographs were taken of the test strips against a black background using a digital camera. Photographs were converted to black and white binary images for quantitative analysis using Image Tool Software (Version 2 alpha, University of Texas Health Science Center, San Antonio, Texas). This software was used to quantify the total spot area (number of black pixels) as a percentage of the total test area (total pixelated area). Sebum outflow was determined as the percentage lipid area (percentage of black area of the whole test area) on Sebutape[®] strips following a 1 hour collection.

5.3.8 *Patient's assessment of skin oiliness*

At baseline and 12 weeks, each participant was asked to rate their facial skin oiliness using a 7-point scale. Responses were reported using a scale from 0 ('not at all') to 6 ('extremely'). This question was a component of an acne-specific questionnaire relating to acne symptoms and quality of life. Details regarding the questionnaire are described elsewhere (Martin *et al.*, 2001).

5.3.9 *Analysis of the fatty acid composition of skin surface triglycerides*

Lipids from the test strip were extracted as previously described (Nordstrom *et al.*, 1986). Triglycerides were isolated by preparative thin layer chromatography using silica 60G plates that had been heated in an oven at 100°C and cooled to room temperature in a desiccator. A reference standard of cholesterol, cholesterol oleate, oleate, methyl oleate, triolein, palmityl oleate and squalene (Nu Chek Prep Inc. Elysian, Minnesota and Sigma chemical Co Missouri) was used to identify the triglycerides and wax ester fractions. After spotting the samples, the plates were developed according to the method of Doran *et al* (1991).

Lipids were visualised on the plates under ultraviolet light after spraying with a methanoic solution of 2',7'-dichlorofluorescein. The bands containing wax esters and triglycerides were isolated and saponified according to the method of Sinclair *et al* (1987). The fatty acid methyl esters (FAME) were analysed by gas chromatography using a Shimadzu GC-17A chromatograph, equipped with a flame ionisation detector and integrated software. Each sample was injected onto a 60m BPX-70 (0.32mm internal diameter and 0.25mm film thickness) bonded phase, fused silica capillary column (SGE, Ringwood, Victoria, Australia) and samples were run according to Sinclair *et al* (1987) using a splitless injection technique. A commercial standard of C14:0, C16:0, C16:1Δ9, C18:0, C18:1Δ9, C18:2Δ9,12, C20:0 (Nu Chek Prep Inc. Elysian, Minnesota) was used for identification of sample FAMEs. Isolated sebaceous wax esters served as a source of identification of 16:1Δ6 (Nordstrom *et al.*, 1986).

5.3.10 *Statistical analysis*

All statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois). Data conforming to a normal distribution were analysed using an independent t test. We analysed ordinal and non-normally distributed data using Mann-Whitney U test for independent groups and the Wilcoxon signed rank test for paired data. Changes from baseline are reported in percentages, with statistical analyses done for absolute values. We compared changes in lesion counts using analysis of covariance of log-transformed data with baseline lesion counts as the covariate. Bivariate linear regression analysis was also conducted, pooling data from both groups, to explore relationships between outcome variables. P -values less than 0.05 were considered significant. As the primary endpoint for this paper was the fatty acid composition of skin surface triglycerides, the data was analysed per protocol.

5.4 RESULTS

5.4.1 Subjects

Figure 5.1 shows the trial profile. Fifty-four subjects were recruited for the parallel, dietary intervention study. Seven participants did not complete the study (five in control and two in LGL), four were removed from data set (two began acne medications and two were non-compliant) and 12 were unable to participate in the sebum test. Thirty-one subjects completed the trial and sebum test as per protocol.

5.4.2 Study outcomes

Table 5.1 shows the clinical characteristics of the subjects who completed the sebum test as per protocol. Baseline characteristics according to group allocation were not significantly different for total ($P=0.15$), inflammatory ($P=0.44$) and non-inflammatory ($P=0.45$) lesion counts. At 12 weeks, a greater improvement in total lesion counts was observed in the LGL group compared to controls after adjusting for baseline differences. Total acne lesion counts decreased by 59% in the LGL group and by 38% in the control group ($P=0.046$). However, there were no significant group differences with regards to changes in inflammatory lesions. The LGL group also showed reductions in weight ($P<0.001$) and BMI ($P=0.001$) when compared to the control group (**Table 5.1**).

Figure 5.2 and **Figure 5.3** illustrate the changing pattern of increasing sebum output and the follicular sebum outflow as determined from the percentage of the lipid impregnated area of test strips. The follicular sebum outflow did not differ between groups at baseline ($P=0.07$) and did not change following 12 weeks of dietary intervention. However, there was a significant decline in the reported oiliness of the skin at 12 weeks in the LGL group (**Figure 5.4**). The mean response score of skin oiliness decreased from baseline in the LGL group ($P=0.013$), whereas no significant change was observed in the control group.

Table 5.1: Clinical outcomes according to dietary group for subjects who completed sebum test as per protocol

| | Baseline | n | 12 weeks | n | Change from baseline ^a | <i>P</i> |
|-------------------------------|-------------------------|----|------------|----|--------------------------------------|----------|
| Total acne lesions | | | | | | |
| LGL | 48.9 ± 5.8 ^b | 16 | 18.9 ± 2.8 | 16 | -59.4 ± 4.6% | 0.046 |
| Control | 38.7 ± 5.1 | 15 | 24.9 ± 4.9 | 15 | -37.8 ± 8.8% | |
| Inflammatory lesions | | | | | | |
| LGL | 38.1 ± 4.7 | 16 | 16.7 ± 2.7 | 16 | -50.0 ± 9.1% | 0.10 |
| Control | 32.1 ± 4.3 | 15 | 22.6 ± 4.3 | 15 | -30.3 ± 10.2% | |
| Weight (kg) | | | | | | |
| LGL | 74.1 ± 2.9 | 16 | 70.5 ± 2.4 | 16 | -4.4 ± 1.0% | <0.001 |
| Control | 72.6 ± 4.0 | 15 | 73.4 ± 4.1 | 15 | 1.0 ± 0.5% | |
| BMI (kg/m²) | | | | | | |
| LGL | 23.0 ± 0.5 | 16 | 21.9 ± 0.4 | 16 | -4.7 ± 1.1% | 0.001 |
| Control | 22.2 ± 0.8 | 15 | 22.3 ± 0.8 | 15 | 0.5 ± 0.6% | |

^a Changes from baseline are reported in percentages, with statistical analyses done for absolute values. Lesion count data was analysed using ANCOVA on log transformed data with baseline counts as the covariate. Body composition data was analysed using Mann-Whitney

^b Results expressed as mean ± SEM. Analysis of baseline data revealed no significant group differences for listed variables.

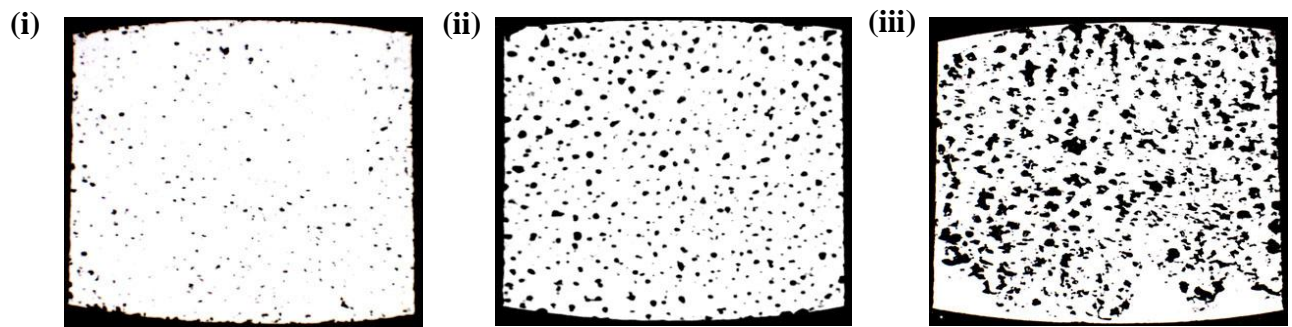


Figure 5.2: Pattern of sebum droplets from subjects exhibiting low (i), moderate (ii) and high (iii) levels of follicular sebum outflow.

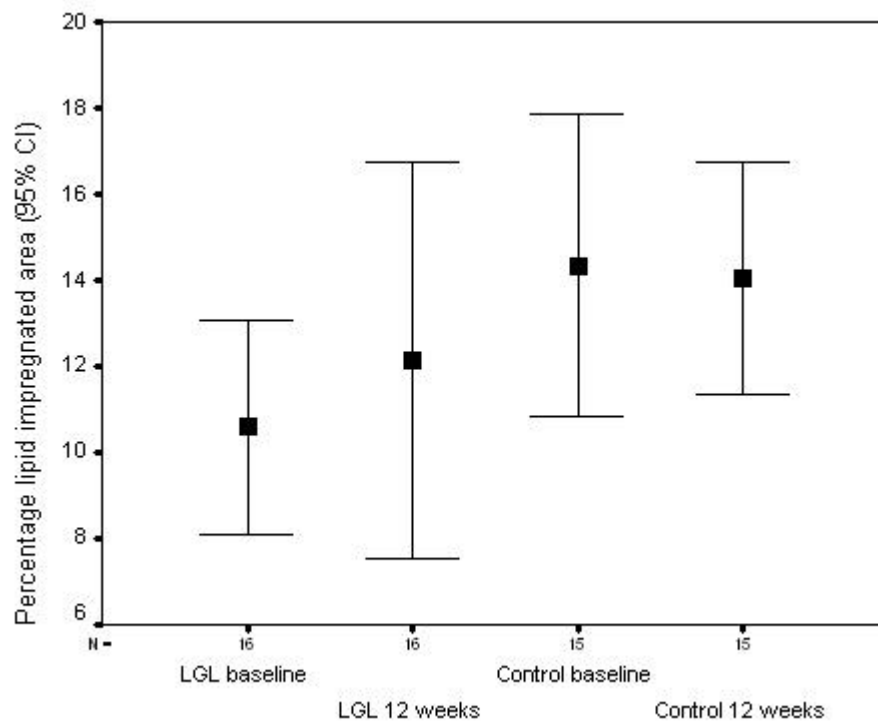


Figure 5.3: Percentage lipid impregnated area (95% CI) of sebutapes at baseline and follow up according to dietary group

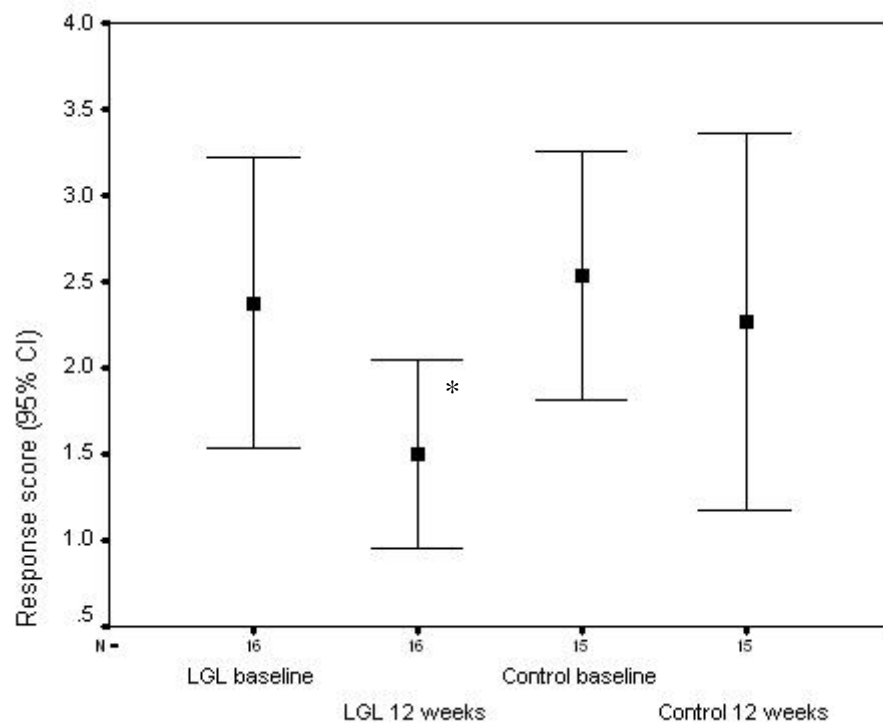


Figure 5.4: Mean response score of skin oiliness (95% CI) according to dietary group at baseline and follow-up.

Note: a lower score reflects a decline in reported skin oiliness

* Follow-up score significantly different from baseline $P < 0.05$

The relative amounts of the major fatty acids in the triglyceride fraction of sebum are summarised in **Table 5.2**. No significant group differences were observed for the change in the proportion of individual fatty acids in sebum. However, the two treatment groups demonstrated opposing trends for changes in the SFAs/MUFAs ratio. The SFAs/MUFAs ratio significantly increased in the LGL group ($P=0.017$) and the change at 12 weeks was significantly different from that of controls ($5.3 \pm 7.8\%$ for the LGL group vs $-2.2 \pm 6.0\%$ for the control group, $P=0.006$). Similarly, the $16:0/16:1_{\Delta6+\Delta9}$ ratio increased in the LGL group compared to the control group ($4.5 \pm 2.3\%$ for the LGL group vs $-5.5 \pm 2.2\%$ for the control group, $P=0.004$).

5.4.3 *Sebacous fatty acids associated with acne improvement and sebum output*

We found that an increase in the SFAs/MUFAs ratio was predictive of the clinical improvement in acne (**Figure 5.5**). Furthermore, an increase in the SFAs/MUFAs was associated with a decline in the follicular sebum outflow (**Figure 5.6**). In light of this, we explored the improvement in acne in terms of changes in sebum output and found that the relationship was not significant ($P=0.70$). However, the sebum outflow was found to be a function of the increasing proportion of MUFAs in sebum (**Figure 5.6**).

Table 5.2: Weights percent of the major fatty acids in the triglyceride fraction of sebum according to dietary group.

| Fatty acid | Baseline | n | 12 weeks | n | Change from baseline ^a | P ^b |
|---|--------------------------|----|-------------|----|-----------------------------------|----------------|
| 14:0 (%wt of total) | | | | | | |
| LGL | 9.22 ± 0.49 ^c | 16 | 9.34 ± 0.35 | 16 | 2.9 ± 2.9% | 0.75 |
| Control | 9.63 ± 0.62 | 15 | 9.62 ± 0.64 | 15 | -0.07 ± 2.4% | |
| 14:1 (%wt of total) | | | | | | |
| LGL | 2.29 ± 0.18 | 16 | 2.24 ± 0.17 | 16 | -3.9 ± 6.2% | 0.65 |
| Control | 2.38 ± 0.11 | 15 | 2.46 ± 0.12 | 15 | 4.3 ± 4.3% | |
| 15:0 (%wt of total) | | | | | | |
| LGL | 6.20 ± 0.32 | 16 | 6.49 ± 0.31 | 16 | 5.4 ± 2.6% | 0.71 |
| Control | 6.75 ± 0.28 | 15 | 6.95 ± 0.40 | 15 | 2.3 ± 2.8% | |
| 16:0 (%wt of total) | | | | | | |
| LGL | 23.3 ± 0.5 | 16 | 23.9 ± 0.5 | 16 | 2.9 ± 2.1% | 0.07 |
| Control | 24.3 ± 0.4 | 15 | 23.6 ± 0.6 | 15 | -2.4 ± 1.7% | |
| 16:1Δ6 + Δ9 (%wt of total) | | | | | | |
| LGL | 19.3 ± 0.6 | 16 | 19.0 ± 0.6 | 16 | -0.7 ± 3.3% | 0.23 |
| Control | 19.2 ± 0.6 | 15 | 19.8 ± 0.5 | 15 | 3.8 ± 2.4% | |
| 18:0 (%wt of total) | | | | | | |
| LGL | 2.85 ± 0.21 | 16 | 2.63 ± 0.13 | 16 | -3.4 ± 3.8% | 0.34 |
| Control | 2.65 ± 0.15 | 15 | 2.62 ± 0.21 | 15 | -0.5 ± 6.7% | |
| 18:2 Δ9,12 (%wt of total) | | | | | | |
| LGL | 0.52 ± 0.06 | 15 | 0.43 ± 0.03 | 15 | -8.9 ± 6.9% | 0.65 |
| Control | 0.43 ± 0.04 | 14 | 0.42 ± 0.06 | 14 | 3.7 ± 15.2% | |
| 20:0 (%wt of total) | | | | | | |
| LGL | 0.53 ± 0.05 | 16 | 0.51 ± 0.04 | 16 | -2.1 ± 6.2% | 0.22 |
| Control | 0.46 ± 0.05 | 15 | 0.47 ± 0.05 | 15 | 4.5 ± 6.3% | |
| Total saturated (%wt of total) | | | | | | |
| LGL | 44.8 ± 0.9 | 16 | 45.6 ± 0.6 | 16 | 2.2 ± 1.4% | 0.09 |
| Control | 46.3 ± 0.8 | 15 | 45.7 ± 0.9 | 15 | -1.4 ± 1.1% | |
| Total monounsaturated (%wt of total)^d | | | | | | |
| LGL | 32.8 ± 1.2 | 16 | 31.8 ± 1.0 | 16 | -2.3 ± 2.6% | 0.15 |
| Control | 32.0 ± 1.0 | 15 | 32.4 ± 0.9 | 15 | 1.7 ± 1.6% | |

^a Changes from baseline are reported in percentages, with statistical analyses done for absolute values

^b P-value corresponds with an independent-sample *t* test or Mann-Whitney for changes in absolute data

^c Results are expressed as mean ± SEM. An independent-sample *t* test revealed no significant group differences for listed fatty acids at baseline.

^d Includes 14:1, 16:1 and 18:1 monounsaturated series.

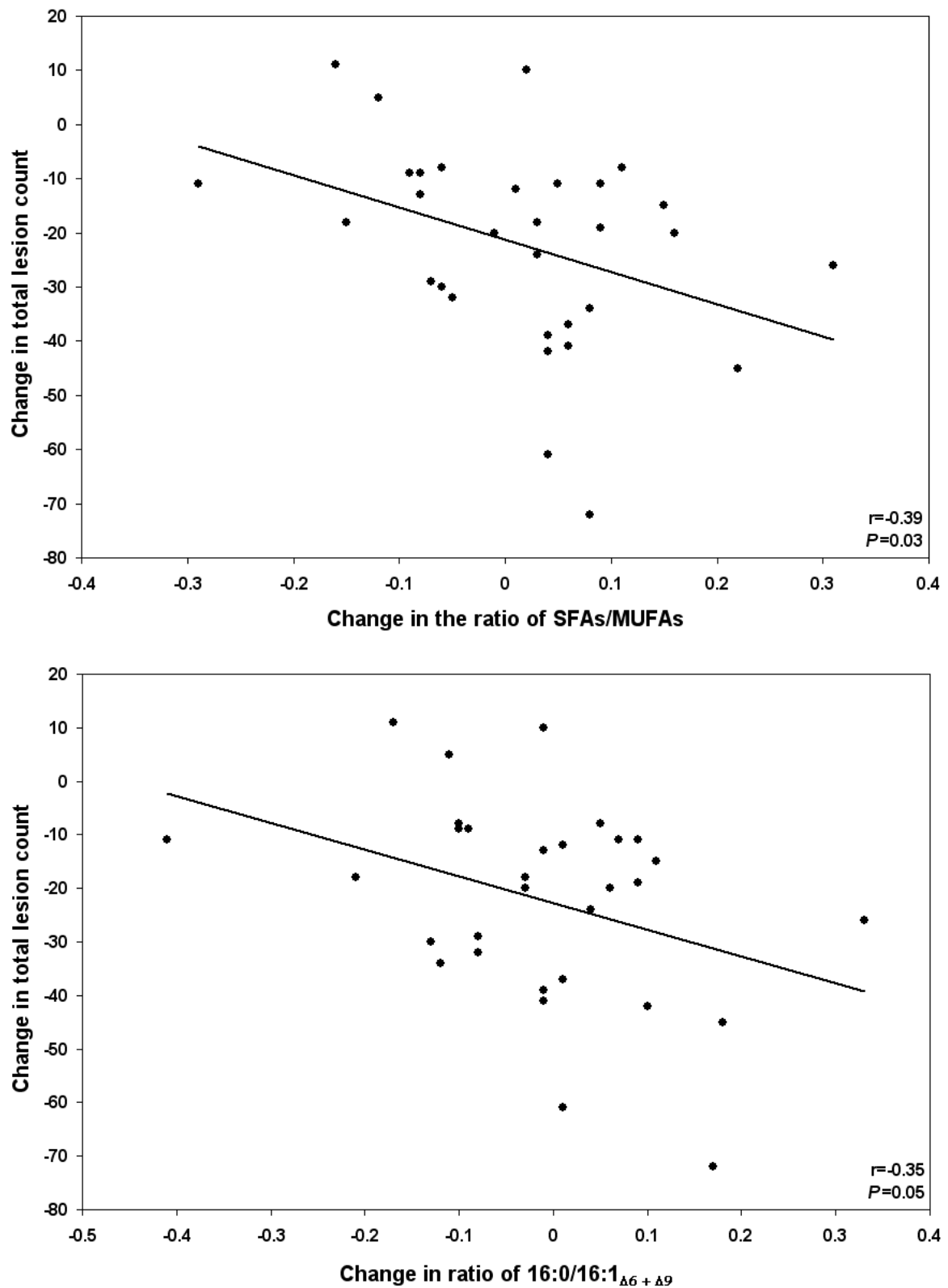


Figure 5.5: (i) Change in acne lesion count as a function of the change in the ratio of SFAs/MUFAs in sebaceous triglycerides. (ii) Change in acne lesion count as a function of the change in the ratio 16:0/16:1_{Δ6 + Δ9} in sebaceous triglycerides.

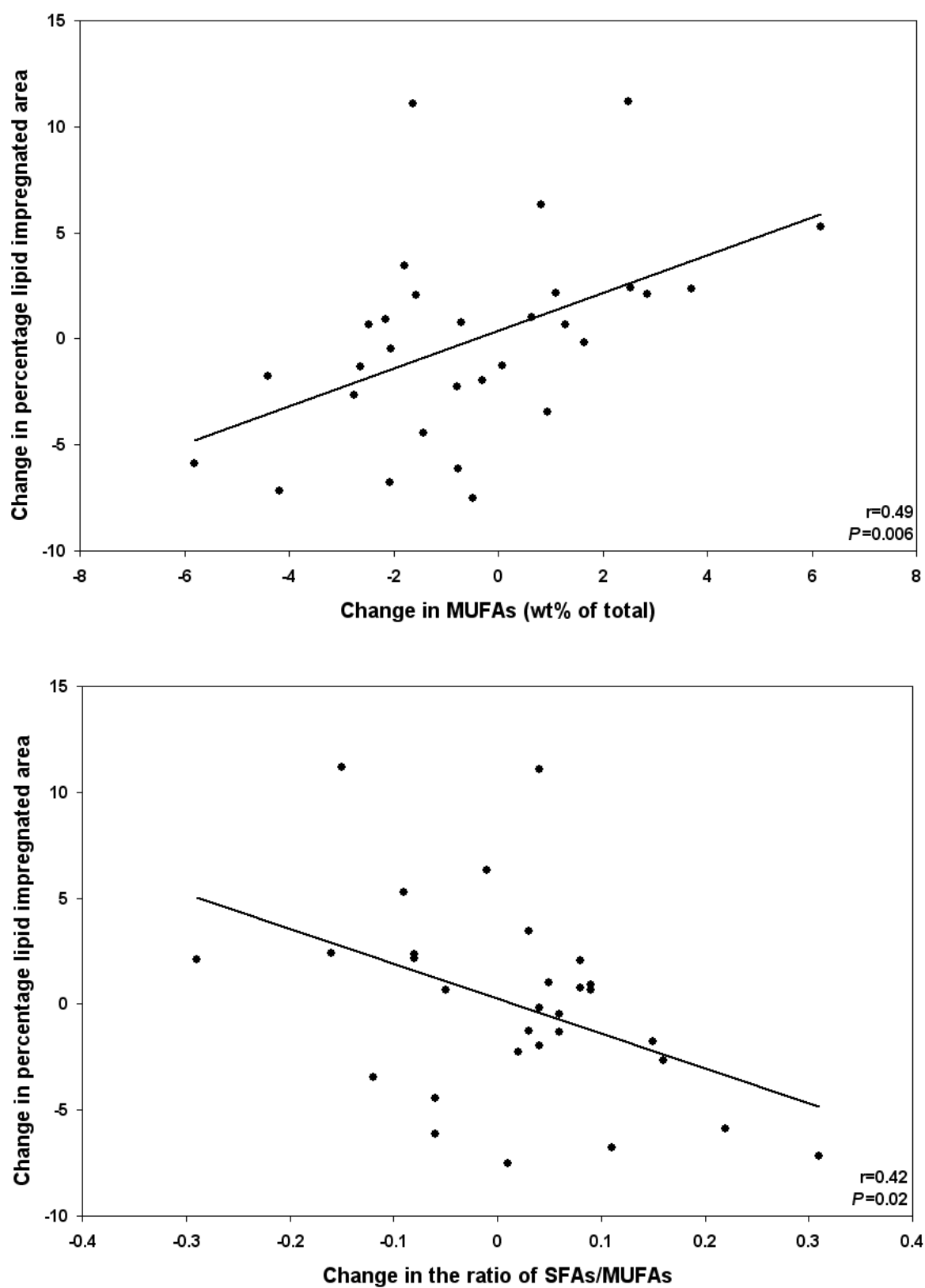


Figure 5.6: (i) Change in the quantity of skin surface lipid as a function of the change in the MUFAs in sebaceous triglycerides. (ii) Change in the quantity of skin surface lipid as a function of the change in the ratio of SFAs/MUFAs.

5.5 DISCUSSION

Increased sebum production is an obligatory condition for developing acne. Support for an association between acne and sebum production comes from three lines of evidence: (i) acne patients have higher rates of sebum secretion than controls (Strauss and Pochi, 1961; Harris *et al.*, 1983); (ii) children have low sebum output and do not get acne; and (iii) sebum-suppressive agents alleviate acne symptoms (Strauss *et al.*, 1962; Farrell *et al.*, 1980). Although the role of sebum in acne remains to be defined, there is suggestive evidence that compositional changes, which occur with increasing sebum production, may influence events involved in comedo formation (Downing *et al.*, 1986). Therefore, profiling the fatty acids of sebum may contribute to our understanding of events involved in acne pathogenesis.

In the present study, we examined the influence of a LGL diet on clinical assessments of acne and the composition of skin surface triglycerides. After 12 weeks, we found that the subjects on a LGL diet demonstrated significantly greater reductions in total lesion counts when compared with controls. Although we were unable to detect an effect of dietary intervention on sebum output or the composition of individual fatty acids, we did observe opposing trends in the SFAs/MUFAs ratio of skin surface triglycerides. Subjects on the LGL diet demonstrated an increase in the SFAs/MUFAs ratio compared to a decrease seen in the control group. The LGL group also demonstrated an increase in the 16:0/16:1_{Δ6+Δ9} ratio, thereby suggesting a decrease in the enzymatic desaturation of 16:0 with a LGL diet. Interestingly, these changes were found to correlate with the clinical improvement in acne, thereby suggesting that the desaturation of sebaceous fatty acids may play a role in acne development.

The enzyme, Δ6-desaturase, is responsible for converting palmitic acid (16:0) into the monounsaturated fatty acid sapienic acid (16:1Δ6) in a unique type of reaction that is characteristic of human sebaceous glands. In sebaceous secretions of other species, the pattern of desaturation occurs at the Δ9 carbon rather than at the Δ6 position (Stewart, 1992). Human sebaceous glands do not express Δ9 desaturase (Ge *et al.*, 2003), however accumulation of some Δ9 forms may occur in undifferentiated cells in the basal layer of the glands. As cells differentiate and move into the unvascularised interior of the gland, the Δ9 fatty acids initially acquired by cells may be diluted by the

subsequent synthesis of $\Delta 6$ fatty acids. In adult sebum, sapienic acid accounts for around 25% of the total fatty acids of sebum and the contribution of fatty acid 16:1 $\Delta 9$ is less than 0.5% (Nicolaidis, 1974). In the present study, we were unable to resolve the $\Delta 6$ and $\Delta 9$ isomers by gas chromatography, however we suspect that basal levels of 16:1 $\Delta 9$ would be fairly constant and therefore any change in 16:1 $_{\Delta 6+\Delta 9}$ is most likely to reflect a change in the endogenous production of 16:1 $\Delta 6$.

The expression of $\Delta 6$ -desaturase and the resultant accumulation of sapienic acid in sebum may be an important factor in sebaceous lipogenesis. Support for this role comes from evidence of sebaceous gland hypoplasia in asebia mice which fail to express $\Delta 9$ desaturase (Zheng *et al.*, 1999). Progenitor cells of the human sebaceous gland do not express $\Delta 6$ -desaturase, however this enzyme is highly expressed in lipid-containing cells situated one cell layer away (Ge *et al.*, 2003). This pattern of expression supports a role of $\Delta 6$ -desaturase in sebaceous lipogenesis and it has recently been proposed that sapienic acid production may provide a functional marker of sebaceous gland activity (Ge *et al.*, 2003). Our study found that a higher follicular sebum outflow was associated with an increase in the proportion of MUFAs in sebum, which was largely explained by variations in 16:1 $_{\Delta 6+\Delta 9}$ (data not shown). This observation supports previous reports of high sapienic acid levels in conditions of increased sebum production (ie. puberty and acne) (Sansome-Bassano *et al.*, 1980; Perisho *et al.*, 1988). We also observed that increased sebum outflow was associated with a decrease in the SFAs/MUFAs ratio, which supports the suggestion that desaturase activity plays a fundamental role in sebum production.

Sebum composition has also been implicated in the abnormal follicular keratinisation which is associated with acne development. Katsuda *et al* (2005) recently demonstrated that fatty acid subtypes can have discrete effects on skin surface morphology and epidermal proliferation. The authors showed that the topical application of MUFAs (oleic acid and palmitoleic acid) induced scaly skin, abnormal keratinisation and epidermal hyperplasia. In contrast, triglycerides (triolein) and SFAs (palmitic and stearic acid) had no affect on skin morphology. It is thought that the MUFAs of sebum interfere with the intracellular calcium dynamics of follicular keratinocytes and the intercellular lipid bilayer structure of the epidermal water barrier. Katsuda *et al* (2005) have postulated that reducing sebum

secretion and/or the production of free MUFAs may serve as potential targets for acne therapy. In support of this hypothesis, our study found that an increase in SFAs relative to MUFAs was predictive of the clinical improvement in acne. This relationship was largely explained by an increase in the 16:0/16:1 $_{\Delta 6+\Delta 9}$ ratio. Given that the LGL group demonstrated an increase in the 16:0/16:1 $_{\Delta 6+\Delta 9}$ ratio when compared to controls, this may serve to explain the greater acne improvement in the LGL group.

The precise mechanism by which dietary glycaemic load influences the sebum composition is unknown. Given that sebaceous glands can synthesise lipids from a variety of precursors, one could assume that the supply of glucose is not a limiting factor. To synthesise lipids, sebaceous glands require energy, acetyl-coenzyme A and NADPH, which can be acquired through β -oxidation of fatty acids and/or the catabolism of glucose (Downie *et al.*, 2004). However, to sustain the normal pattern of sebaceous lipids, Downie and Kealey (2004) have hypothesised that endogenous glycogen may be an important provider of NADPH, as well as substrates (eg. glycerophosphate and acetate), for the synthesis of triglycerides. Glycogen and glucose concentrations have been shown to decrease as cells accumulate lipid and move from the periphery to the centre of the glands (Im and Hoopes, 1974; Downie and Kealey, 1998). The glycogen content of undifferentiated sebaceous cells is also 6.3 times that of the epidermal keratinocytes (Im and Hoopes, 1974). Furthermore, glycogen stores are greatly reduced in isolated sebaceous glands, even in the presence of acetate, lactate and amino acid substrates, thereby suggesting that endogenous glycogen is essential for sebaceous lipogenesis (Downie and Kealey, 1998).

We have hypothesised that a LGL diet may affect sebum composition via metabolic effects (eg. fuel storage) and/or secondary effects on hormone levels (eg. free testosterone and adrenal androgens) (see **Figure 5.7**). Accumulating evidence suggests that high glycaemic load diets can increase glycogen storage within body tissues (ie. muscle, liver) when compared to isoenergetic, low glycaemic load diets (Kiens and Richter, 1996; Brand-Miller *et al.*, 2002). Therefore, it is possible that dietary manipulation of the glycaemic load may affect glycogen stores in sebaceous glands, which may be a limiting and directing factor in sebaceous lipogenesis. Furthermore, we have shown that a LGL diet can reduce testosterone bioavailability and dehydroepiandrosterone sulfate concentrations (Smith *et*

al., 2007), which may be explained by the insulin lowering effect of these diets (Cordain *et al.*, 2002). As sebum production is largely under androgenic control, a reduction in circulating androgen levels may also account for the changes in sebum composition. This raises the possibility that nutritional intake may influence sebum production via the synchronised modulation of androgens and glycogen stores.

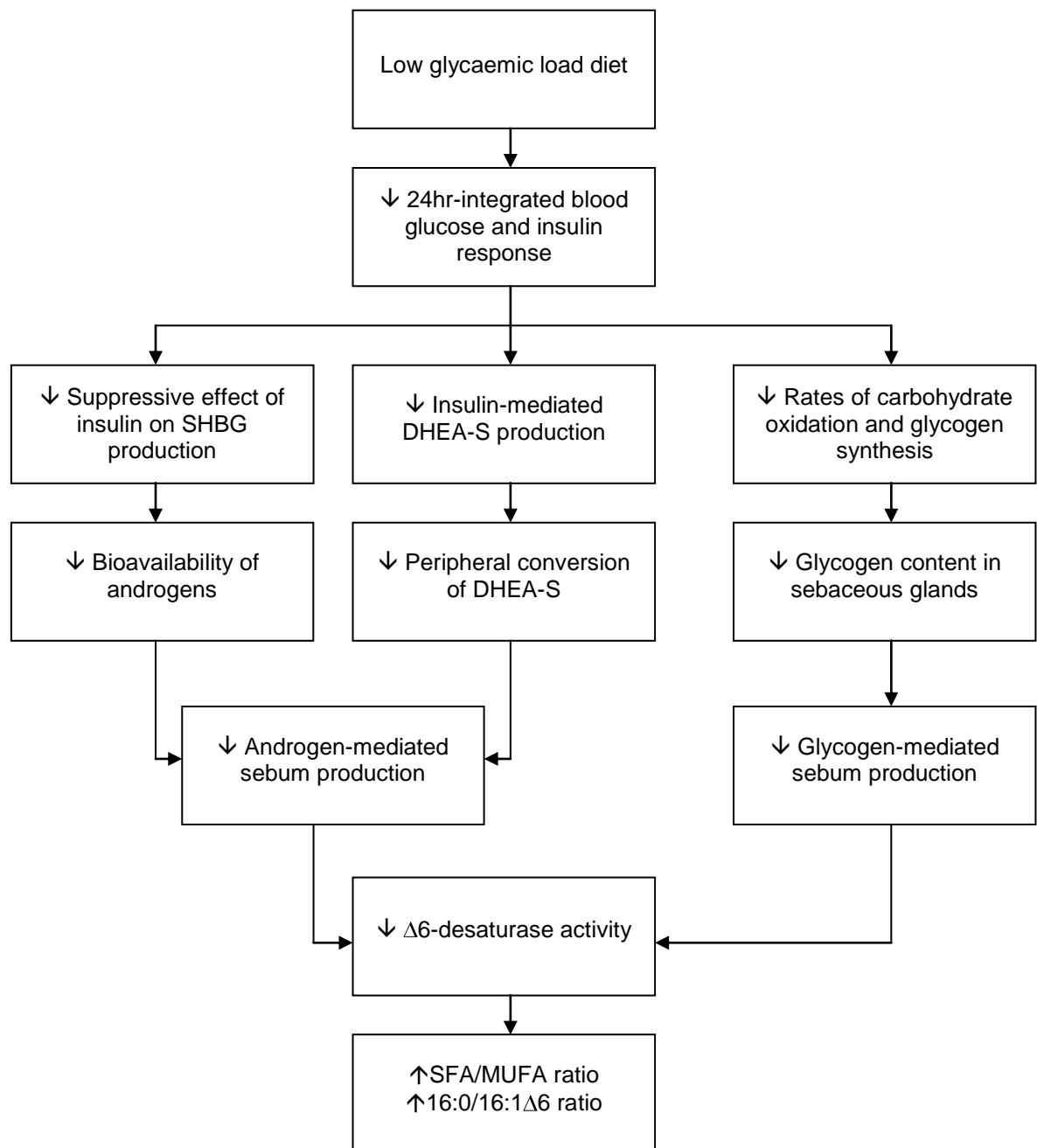


Figure 5.7: Schematic illustrating the hypothesised metabolic-endocrine pathway linking a low glycaemic load diet and the changes in the fatty acid profile of human sebum production.

There are some issues relating to this study that warrant further consideration. Firstly, it is possible that the relative changes in sebum composition could also be related to the change in body mass in the LGL group (Tsai *et al.*, 2006). This weight loss occurred despite dietary instructions being given to maintain the subject's baseline kilojoule intake. There is increasing evidence to indicate that LGL diets enhance weight loss via increases in satiety and fat oxidation (Kiens and Richter, 1996; Brand-Miller *et al.*, 2002), and studies suggest that obese adolescents lose weight on LGL diets without the need for an imposed energy restriction (Spieth *et al.*, 2000; Ebbeling *et al.*, 2003). This study found that the weight loss in the LGL group correlated with starting BMI, indicating that extent of obesity at baseline was a significant predictor of the overall weight loss (data not shown). Secondly, it is possible that use of the mild non-comedogenic skin cleanser may have assisted in the management of acne-associated symptoms (Draelos, 2006). Although the participants were stabilised on the mild cleanser two weeks prior to baseline, the acne improvement seen in controls suggests a possible therapeutic effect of the cleanser use. However, as the cleanser was standardised for both groups, this would not explain the differences observed between groups.

In conclusion, this study is the first to report the effect of a LGL diet on follicular sebum outflow and composition of skin surface triglycerides. We observed that after 12 weeks a LGL diet reduced weight and total lesion counts when compared to controls. The LGL group also demonstrated a significant increase in the ratio of SFAs/MUFAs, which was largely explained by alterations in the ratio of 16:0/16:1_{Δ6+Δ9}. These changes suggest either a decrease in desaturase activity or the levels of the desaturase enzyme. As changes in the ratio of 16:0/16:1_{Δ6+Δ9} correlated with the clinical improvement in acne, the desaturation of 16:0 may have practical implications for studying the disease process. However, as the participants in the LGL group lost weight we are unable to isolate the independent effects of dietary composition from that of weight loss. Therefore, the role of diet in sebum composition is yet to be fully clarified and further studies are required to isolate the underlying mechanistic factors.

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CHAPTER SIX

Evaluating the effect of a low glycaemic load diet on Acne-Specific Quality of Life parameters

6.1 ABSTRACT

Background: Although knowledge concerning the negative psychological effects of acne has grown in recent years, relatively few studies have assessed the impact of treatment upon the emotional and functional status of a patient. The Acne-Specific Quality of Life (Acne-QoL) questionnaire was purposely developed to assess the impact of treatment in acne clinical trials.

Objective: To evaluate the effect of dietary treatment on Acne-QoL

Methods: Participants self-completed the Acne-QoL questionnaire at baseline and at study exit as part of a 12 week, dietary intervention trial comparing a low glycemic load diet with a conventional high glycemic load diet. The Acne-QoL instrument is designed to measure the impact of acne across four domains of patient QoL (self-perception, role emotional, role social, acne symptoms). Domain score changes with this instrument were used to report treatment advantages following dietary intervention. Higher scores reflect more favourable improvements in Acne-QoL.

Results: At 12 weeks, the total Acne-QoL score (mean \pm SEM) increased significantly more ($P=0.001$) in the low glycaemic load group (16.8 ± 5.6) when compared with controls (-0.2 ± 3.2). The low glycemic load group showed greater improvements in the self-perception (5.4 ± 1.2 compared with 0.9 ± 0.9 ; $P<0.01$), role social (2.6 ± 0.9 compared with -2.2 ± 1.27 ; $P<0.01$), and role emotional (5.0 ± 1.3 compared with -0.3 ± 0.9 ; $P<0.01$), domains. However, no significant group differences were observed for the change in the acne symptoms domain score.

Conclusion: These results suggest that patient-assessed outcomes respond to diet-mediated improvements in acne, suggesting that the emotional, social and psychological disability of acne may be partially improved with a low glycemic load diet.

6.2 INTRODUCTION

Although acne is not a life threatening condition, the physical and psychological morbidity associated with the disease is well recognised (Kilkenny *et al.*, 1997; Niemeier *et al.*, 2002; Yazici *et al.*, 2004). Physical morbidity relates to acne type (comedones, papules and pustules), distribution (face, chest, back) and extent (number of lesions). As acne often affects the face, significant psychological morbidity can arise from the perceived disfigurement of one's appearance (Henkel *et al.*, 2002). It is estimated that 30-50% of 12-20 year olds with facial acne show negative psychological symptoms, including anxiety, depression, self-consciousness, embarrassment, diminished self-esteem, as well as perceived social rejection (Gupta *et al.*, 1990; Gupta and Gupta, 1998; Aktan *et al.*, 2000; Smithard *et al.*, 2001). These factors may contribute to lower academic performance, higher unemployment and problems in interpersonal relationships (Jowett and Ryan, 1985; Cunliffe, 1986; Girman *et al.*, 1996). Therefore, if untreated, acne can impose a negative impact on the psychological development of adolescents and young adults and can significantly diminish an individual's quality of life.

Effective treatment can potentially reverse some of the negative psychological effects of acne (Newton *et al.*, 1997). Several studies indicate that amelioration of acne can decrease symptoms of depression and anxiety and improve other psychosocial parameters (Rubinow *et al.*, 1987; Myhill *et al.*, 1988; Newton *et al.*, 1997; Weiss *et al.*, 2003). In fact, one study found that patients who achieved the greatest clinical improvement in acne also experienced the greatest reduction in negative psychological symptoms (Rubinow *et al.*, 1987). However, this is not always the case as some patients maintain the psychological response to the disease, even after successful treatment (Kellet and Gawkrödger, 1999). The negative psychological impact of acne is highly individualistic and emotional impairment does not always correlate with the objective severity of the disease (Niemeier *et al.*, 1998). Therefore, patient care should focus on improving both the clinical symptoms and the experience of the disease (Newton *et al.*, 1997).

Over recent years, several quality of life tools have been developed to inform clinicians about the impact of disease on individuals. Generic instruments, such as the General Health Questionnaire (GHQ-28) and

the Short Form 36 (SF36), provide meaningful information on aspects of disability or well-being without reference to the cause. Using these scales, one study found that the social, psychological and emotional impact of acne was as great as that reported by patients with chronic disabling diseases such as asthma, epilepsy, diabetes, back pain or arthritis (Mallon *et al.*, 1999). However, generic instruments often provide inconsistent results, in part because these scales are developed for assessing general health status, and do not address problems which are relevant to the disease of interest (Finlay, 2004). Disease-specific quality of life instruments, such as the Cardiff Acne Disability Index and the Acne-Specific Quality of Life Questionnaire, have a greater power to detect change following therapy as they focus on aspects that are most affected by the disease and tend to be of greatest importance to patients (Finlay, 2004). As disease-specific quality of life tools are more sensitive to change than generic instruments, they can be utilised in clinical trials to discriminate between treatments.

The following chapter describes the use of an Acne-Specific Quality of Life (Acne-QoL) tool to evaluate the efficacy of dietary treatment of acne from the patient's perspective. We have previously described that a low glycaemic load (LGL) diet significantly improved objective measures of acne when compared to controls; however, the patient's experience of the disease from a personal and social context has not been described. This chapter also describes the reliability, validity and responsiveness of the Acne-QoL instrument for psychometric evaluation.

6.3 METHODS

The Acne-Specific Quality of Life (Acne-QoL) tool was incorporated as part of the 12 week, parallel, dietary intervention study to evaluate participant satisfaction with dietary treatment. Subjects self-completed the Acne-QoL questionnaire during visits to the academic research clinic at baseline and the study's termination. A discrete group of acne sufferers (n=4) also completed the questionnaire twice in one week to assess the reproducibility of the questionnaire. Details regarding the study design and population have been reported elsewhere (Smith *et al.*, 2007a).

Table 1 provides a summary of the content areas of the Acne-QoL questionnaire and a full description is provided by Martin *et al* (2001). Although the Acne-QoL tool was initially validated within a usual care setting, a subsequent study demonstrated that the questionnaire is capable of detecting treatment differences in randomised clinical trials (Fehnel *et al.*, 2002). The Acne-QoL questionnaire is composed of 19 items, each referring to the past week, which may be organised into four domains: self-perception, role-emotional, role social and acne symptoms. Responses to each item are scored using a 7-point likert scale from 0 ('extremely' or 'extensive') to 6 ('not at all' or 'none'). For each domain, scores are calculated by summing the responses within each category to yield four domain scores, and higher scores indicate more favourable QoL.

This study evaluated the Acne-QoL instrument to ensure that items are answered consistently by respondents (instrument reliability) and to confirm that Acne-QoL scores are sensitive to clinical parameters (instrument validity). The instrument reliability of the Acne-QoL instrument was assessed according to the internal consistency of items and by the test-retest method. Internal consistency was assessed using Cronbach's alpha coefficient, which examines the consistency of responses to items within each domain. Cronbach's alpha values between 0.70 and 0.95 suggest that scores are reliable (Streiner, 1993). The test-retest reliability coefficient was determined by administering the same questionnaire one week later to the small group of volunteers (n=4) and correlating the two sets of scores. A test-retest correlation coefficient value greater than 0.75 is considered acceptable (Martin *et al.*, 2001). Convergent and divergent validity was evaluated by correlating each of the four Acne-QoL domains scores with clinical measures that were presumed to be related (acne severity) and unrelated to Acne-QoL (height and HDL cholesterol). A priori assumption was that height and HDL cholesterol are not related to acne-specific measures of QoL.

The Acne-QoL instrument was assessed for internal responsiveness ('ability to measure change over time') and external responsiveness ('the extent to which changes in a measure over time relate to corresponding changes in a reference measure of health status') as previously described (Husted *et al.*,

2000). Internal responsiveness was assessed using the paired t -test (separately for the LGL and control groups) and the Guyatt's internal responsiveness statistic, which is defined as the difference in average change scores between the two treatment groups, divided by the standard deviation of changes scores in the control group (Guyatt *et al.*, 1987). External responsiveness was evaluated using Pearson's product-moment coefficient for the relationship between the change in Acne-QoL and clinical improvement in acne. Treatment differences were determined by comparing the change in Acne-QoL scores at the end of the 12 week study using an independent t -test or the Mann-Whitney U test. P values less than 0.05 were considered significant.

Table 6.1: Summary of Acne-QoL domains

| Self-perception | Role-social | Role-emotional | Acne symptoms |
|-----------------------------|-------------------------------|-----------------------------|----------------------------------|
| –Feeling unattractive | –Concern about going out in | –Annoyed about time spent | –Bumps on face ^a |
| –Feeling self-conscious | public | cleansing/treating face | –Bumps full of pus ^a |
| –Diminished self-confidence | –Concern about meeting new | –Concern about need to have | –Scabbing from acne ^a |
| –Feeling embarrassed | people | medication/cover up | –Concern about scarring |
| –Dissatisfaction with self- | –Problems associated with | available | –Oily skin |
| appearance | interacting with the opposite | –Concern about not looking | |
| | sex | one’s best | |
| | –Problems associated with | –Feeling upset about having | |
| | socialising with people | facial acne | |

All questions refer to the subject’s experience with facial acne in the past week

Responses to all but three questions are scaled: extremely, very much, quite a bit, a good bit, somewhat, a little bit, and not at all.

^aThe response options for these questions includes: extensive, a whole lot, a lot, a moderate amount, some, very few, and none.

6.4 RESULTS

6.4.1 *Instrument reliability*

Table 6.2 shows the reliability coefficients for the Acne-QoL instrument. The internal consistency of items within Acne-QoL domains was high, suggesting good agreement among items within each domain. Although the coefficient of test-retest reliability was computed on a smaller than ideal sample ($n=4$), the correlation was high for the self-perception, role emotional and role social domains. However, the repeated test scores for the acne symptoms domain only achieved a satisfactory correlation (see **Table 6.2**).

6.4.2 *Instrument validity*

The strong correlation of worsening acne symptoms scores with increasing acne severity (total lesion count and severity rating) supports the convergent validity of the Acne-QoL construct, demonstrating that objective clinical assessments of acne correlated with the respondent's rating of physical symptoms. A somewhat weaker association was shown for the role emotional and self perception domains ($r = 0.17-24$) and no relationship was observed with the role social domain. However, the latter scales measure the emotional and social impact from all sources and are therefore less specific to the disease. Discriminant validity was also supported by the very low correlations obtained between the Acne-QoL domain scores and clinical measures assumed to be unrelated to acne-specific QoL (height and HDL cholesterol).

6.4.3 *Internal and external responsiveness of the Acne-QoL tool*

Figure 6.1 illustrates the mean change over time in Acne-QoL domain scores for the LGL and control groups. The LGL group demonstrated significant increases across all four domain scores at 12 weeks, suggesting improvements in acne-specific QoL for subjects on the LGL diet. In contrast, Acne-QoL domain scores did not significantly change over time in the control group. The Guyatt's responsiveness statistic (see **Table 6.4**) was moderate-high for all domains, suggesting that the Acne-QoL tool was adequately responsive to measure treatment advantages (ie. the degree of variability in the general population was low compared to the difference in the mean change scores between the LGL and

control groups). Taken together, these results provide strong evidence for the instrument's internal responsiveness or ability to detect change.

Table 6.3 demonstrates how changes in Acne-QoL domain scores and clinical assessments of acne relate. Acne improvement, defined as either a reduction in lesion counts or global severity, appropriately correlated with improvements in the Acne-QoL acne symptoms score, indicating that the respondent's subjective rating of acne improved proportionally to the physician's assessment of acne improvement. Although similar trends were observed for the other domains, their relationship with the physician's clinical assessments did not meet statistical significance. However, a reduction in total lesion counts was found to correlate with the improvement in the overall Acne-QoL domain score.

6.4.4 *Treatment differences in Acne-QoL*

Statistically significant 'between group' differences were observed for changes in the self-perception, role social and role emotional domains, with greater improvements in average Acne-QoL domain scores for the LGL group compared to controls (see **Table 6.4**). However, no significant group differences were observed for the change in the acne symptoms domain score.

Table 6.2: Reliability and validity of the Acne-QoL instrument

| Domain | Reliability | | Validity ³ | | | |
|-----------------|-----------------------------------|--------------------------|-----------------------|-----------------|--------|-----------------|
| | Internal consistency ¹ | Test-retest ² | Total lesion count | Severity rating | Height | HDL cholesterol |
| Self perception | 0.91 | 0.93 | -0.17 | -0.17 | -0.04 | -0.15 |
| Role social | 0.89 | 0.94 | 0.05 | 0.02 | 0.05 | 0.00 |
| Role emotional | 0.83 | 0.82 | -0.24 | -0.23 | 0.04 | -0.08 |
| Acne symptoms | 0.70 | 0.53 | -0.54 | -0.52 | 0.06 | 0.02 |

¹ Cronbach's alpha coefficient for internal consistency of items within a domain, n=43

² Pearson's correlation coefficient using the test-retest method obtained within one week, n=4

³ Pearson's correlation coefficients of clinical measures that are assumed to be both related (lesion counts, severity rating) and unrelated (height and HDL) to Acne-QoL, n=43

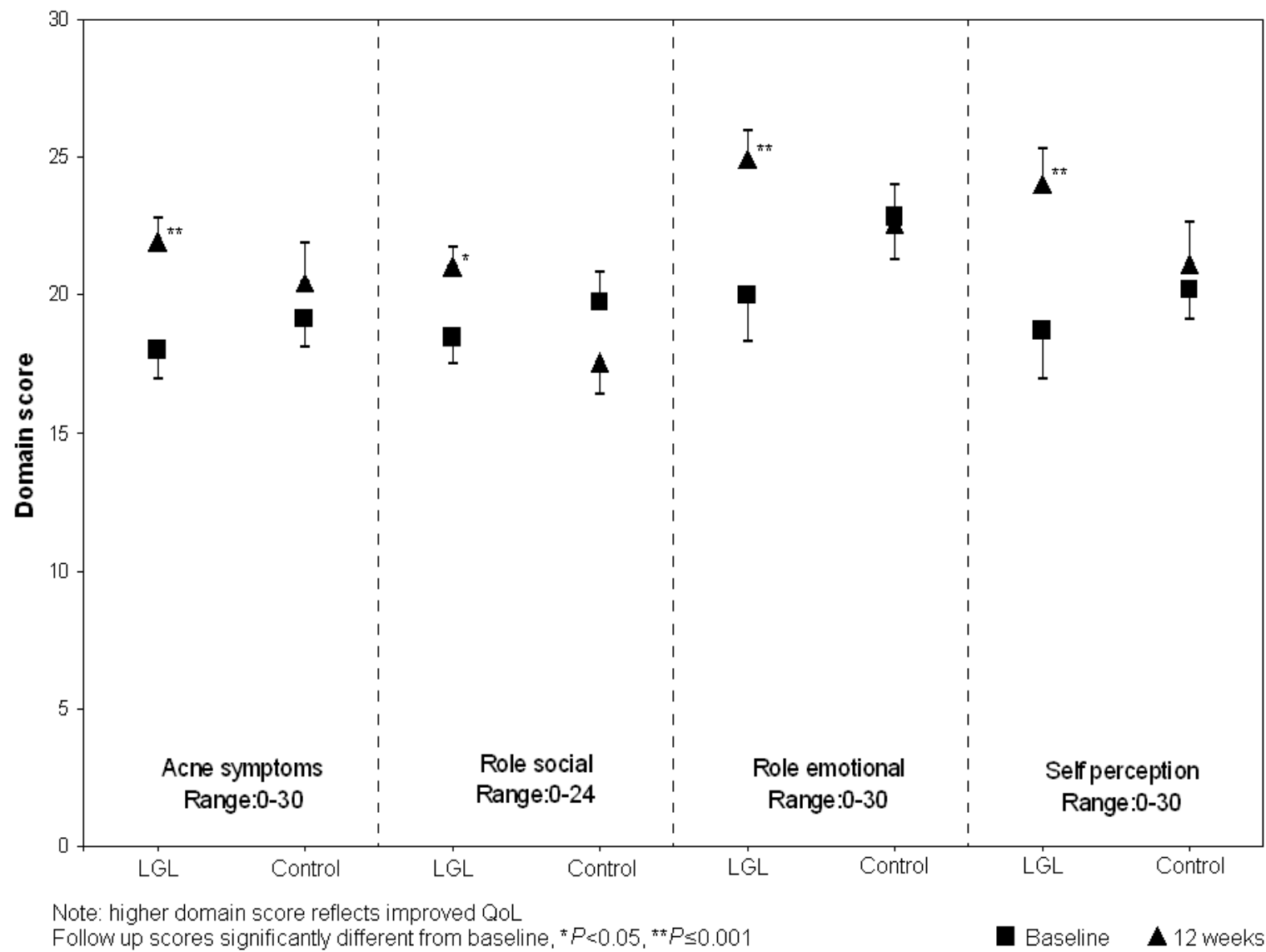


Figure 6.1: Mean domain scores (+/- SEM) according to dietary group at baseline and 12 weeks

Table 6.3: Correlation of Acne-QoL change scores against the objectively assessed improvement in clinical symptoms

| Domain | Total lesion count | Severity rating ¹ |
|-----------------|---------------------|------------------------------|
| Self-perception | -0.21 ² | -0.20 |
| Role-emotional | -0.27 | -0.15 |
| Role-social | -0.16 | -0.22 |
| Acne symptoms | -0.41 ^{**} | -0.38 [*] |
| Total score | -0.31 [*] | -0.28 |

¹ Severity rating according to Cunliffe-Burke global method

² Pearson's correlation coefficient

* $P < 0.05$, ** $P < 0.01$

Table 6.4: Mean change in domain scores from baseline¹ according to dietary group allocation

| Domain | LGL group (n=23) | | Control group (n=20) | | Guyatt's effect size ² | P^3 |
|-----------------|------------------|------|----------------------|------|-----------------------------------|-------|
| | Mean | SEM | Mean | SEM | | |
| Self-perception | 5.35 | 1.23 | 0.90 | 0.94 | 1.06 ⁴ | 0.007 |
| Role-emotional | 4.96 | 1.33 | -0.30 | 0.87 | 1.35 | 0.003 |
| Role-social | 2.57 | 0.92 | -2.15 | 1.27 | 0.83 | 0.004 |
| Acne symptoms | 3.89 | 0.95 | 1.35 | 0.99 | 0.57 | 0.07 |
| Total score | 16.76 | 3.56 | -0.20 | 3.2 | 1.18 | 0.001 |

¹ A higher domain scores reflects improved QoL

² Guyatt's index defined as the difference in average change scores between the two treatment groups, divided by the standard deviation of change scores in the control group.

³ Between group differences for the change in domain scores

⁴ Effect sizes near 0.20 represent small effects, those near 0.50 represent moderate effects and those near 0.80 represent large effects (Cohen, 1977).

6.5 DISCUSSION

In the past, studies comparing therapeutic interventions for acne have focused on clinical outcomes, with little attention given to the impact of treatment on the individual's health-related QoL. This is because laboratory and clinical measures tend to perform better in terms of sensitivity and reliability than QoL measures (Newton *et al.*, 1997). However, the advent of validated disease-specific tools, such as the Acne-QoL instrument, has made these measures more reliable and more sensitive to treatment when compared to instruments which are not specific to the disease (Girman *et al.*, 1996). The results of the present study confirmed that the Acne-QoL tool is both a reliable (as assessed from internal consistency and test retest methods) and valid tool (as determined from convergent/divergent validity tests) for psychometric evaluation.

This study found that not all Acne-QoL domains cross-sectionally correlated with clinical parameters, presumably because the Acne-QoL tool is designed to measure health-related QoL, rather than disease severity. In the present study, Acne-QoL domains were found to strongly correlate with clinical parameters if they contained questions which explicitly relate to symptoms of acne (such as the acne symptoms domain). In contrast, weak relationships were observed between disease severity and domains that measure the psychosocial impact of acne (such as the role social and role emotional domains). This is likely to be because the latter scales are less specific to the disease and may relate more to the individual and their environment. The impact of acne on a given individual can be influenced by several factors, including age and gender, baseline self esteem, response and support of family and friends, personality and coping mechanisms (Baldwin, 2002; Fried and Wechsler, 2006). Furthermore, Acne-QoL can also be influenced by underlying psychiatric disorders that may predate the onset of acne (Baldwin, 2002).

This study demonstrated that the Acne-QoL tool is sensitive to change and is capable of detecting treatment differences. We have previously described that a low glycaemic load diet significantly improved clinical parameters of acne when compared to controls (Smith *et al.*, 2007a). In addition to these clinical improvements, subjects on the LGL diet demonstrated statistically significant treatment

advantages across all four aspects of health-related QoL (self-perception, role social, role emotional and acne symptoms). Of the four domains, a clear relationship was observed between the change in the Acne-QoL acne symptoms score and the improvement in measured clinical parameters (eg. total lesion counts and acne severity), indicating that the respondent's subjective rating of acne symptoms improved proportionally to the objective clinical measurement of the physician (**Table 6.3**). However, changes in the other three domains, self-perception, role social and role emotional, did not correlate with the clinical improvement, possibly because the psychological impact is highly individualistic and can relate to factors other than disease severity (Niemeier *et al.*, 1998). For instance, some individuals with mild acne may report highly negative on QoL scales, while others with severe acne may not. Previous studies have found little correlation between acne severity and psychological measures (Motley and Finlay, 1989; Chee-Chong *et al.*, 1991; Krowchuk *et al.*, 1991), while others found that QoL correlates more closely with patient-reported disease severity than with physician-reported severity (Martin *et al.*, 2001). Similarly, our study found that an improvement in the acne symptom domain score (a measure of self reported disease severity) was significantly associated with improvements in the role emotional, role social and self perception domains (results not shown). These findings suggest that the psychosocial impact of the disease may be more sensitive to self-image, than to the objective clinical measurements.

Although subjects on the LGL diet demonstrated greater improvements in Acne-QoL domains when compared to controls (Table 6.4), it is difficult to determine whether these treatment differences are clinically meaningful. Because Acne-QoL measures aren't as familiar as common clinical measures (ie blood pressure), the minimally reported clinical difference (MCID) was established to help interpret QoL change scores (McLeod *et al.*, 2003; Terwee *et al.*, 2003). In general, MCID refers to the 'smallest difference in QoL domains that can be judged as worthwhile when assessing the effect of treatment' (Terwee *et al.*, 2003). McLeod *et al.* (2003) previously demonstrated that Acne-QoL score changes of 5.2, 4.7, 3.1 and 4.6 or more for the self-perception, role-emotional, role-social and acne symptoms domains, respectively, corresponded with a change in acne which was considered important to patients. According to these estimates, this would suggest that participants on the LGL diet demonstrated clinically meaningful improvements in the self-perception (5.4) and role-emotional (5.0)

domains, but not the role-social (2.6) and acne symptoms (3.9) domains (**Table 6.4**). However, the preferred methodology for defining MCID has not yet been established, as MCID estimates may be inflated when using a patient's assessment of the improvement as opposed to the physician's. Furthermore, it can be concluded that the definition of an important change depends on the study-specific judgement of participants (Terwee *et al.*, 2003). Therefore, the MCID estimates suggested by McLeod *et al* (2003) are only to be used as a guide and further research is needed to determine Acne-QoL change scores that are clinically meaningful.

These results provide further support for a role of low glycaemic load diets in the management of *acne vulgaris*. However, the generalisability of these results should be considered, as this study only included males with mild-moderate acne. Females may psychologically respond differently to dietary interventions as the psychological impact of acne can be greater in females than in males (Krowchuk *et al.*, 1991; Kellet and Gawkrödger, 1999). Furthermore, certain patients that are designated as having severe acne or being at “high risk” of depressive symptoms may warrant a more aggressive therapeutic plan in order to avert negative outcomes (Baldwin, 2002). Dietary measures may have limited potential in this regard and in these cases therapeutic agents may be required.

There were a small number of limitations of this study that should be discussed. Firstly, as the Acne-QoL questionnaires were administered in the research clinic, participant's responses may be biased by what participants think staff might expect them to say. However, we chose this option because response rates are generally low with a silent mail system. In addition, this study only assessed negative components of acne-specific QoL and did not assess negative experiences with following a diet. Diet is an especially difficult aspect of any treatment regimen and lower adherence rates are generally found with diets when compared with pharmaceutical agents (Ahlgren *et al.*, 2001). However, this study found good compliance according to self-report and objective physiological measures (Smith *et al.*, 2007a; Smith *et al.*, 2007b), suggesting that the diets were well tolerated.

The results reported here suggest that patient-assessed outcomes respond to diet-mediated improvements in acne, suggesting that the emotional, social and psychological disability of acne at

least be partially alleviated with a LGL diet. The World Health Organisation currently defines health as not merely the absence of disease or infirmity, but the ability of a person to lead a productive and enjoyable life (World Health Organisation, 1997). This definition, which has been instrumental in defining health-related QoL, acknowledges the potential discordance between clinical severity and the impact of the disease. Although our study found that patient outcomes improved with a LGL diet, the objective severity of acne did not predict the improvement in psychosocial factors. We suspect that LGL dietary interventions may affect Acne-QoL primarily in terms of physical symptoms and this, in turn, may have patient-specific effects on social functioning and general psychological distress. These results further support a role of diet in the management of acne, as patient preferences and health-related QoL are important considerations in clinical decision making.

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CHAPTER SEVEN

Acne in adolescence

A role for nutrition?

Robyn Smith¹ and Neil Mann^{1,2}

¹School of Applied Sciences, RMIT University, Melbourne, Australia

²Australian Technology Network, Centre for Metabolic Fitness

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During the past century, much controversy has surrounded the subject of diet in acne management. In the 1930s, major dermatology textbooks advocated dietary restrictions for acne patients based upon “clinical experience.. that [suggests] a diet high in carbohydrates and sweets seems to make some acne cases worse” (Wise and Sulzberger, 1933). However, apart from the individual impressions of physicians, there was little evidence to support dietary practices for the control of acne. Forty years later, dietary advice was removed from standard texts and the consensus has since been that “diet plays no role in acne treatment in most patients” (Kaminester, 1978). Nonetheless, many acne patients continue to believe that acne is exacerbated by diet (Rasmussen and Smith, 1983; Tan *et al.*, 2001) and a recent survey found that 41% of final year medical students at Melbourne University regarded diet as an aggravating factor (Green and Sinclair, 2001).

Few studies have examined the diet and acne connection and those that have, have design faults. Fulton *et al* (1969) in a crossover single-blind study found no effect of chocolate on acne when compared to a placebo bar. However, a later examination of the ingredients in the placebo bar indicated that the fatty acid composition and sugar contents were virtually identical to that found in the experimental treatment (Mackie and Mackie, 1974). Anderson (1971) examined the effect of the daily consumption of chocolate, milk or nuts and found no effect on acne. However, the present study has been criticised for its small sample size (minimum of 3 subjects for each food), the short follow-up and its lack of controls (Treloar, 2003). Although the experiments of Fulton *et al* (1969) and Anderson (1971) are frequently cited as evidence that diet plays no role in acne development, a major limitation of both studies was that they concentrated on single foods with no background dietary analysis.

Variations in acne prevalence worldwide have prompted researchers to question the natural development of the disease. For instance, rates of acne in Kenya (Verhagen *et al.*, 1968), Zambia (Ratnam and Jayaraju, 1979) and Peru (Freyre *et al.*, 1998) are reportedly lower than in the black (Wilkins and Voorhees, 1970; Taylor *et al.*, 2002) and Hispanic (Taylor *et al.*, 2002) populations of Western countries. In a recent observational report, Cordain *et al* (2002) attributed that the absence of acne in two non-Westernised societies to environmental factors, mainly local diets, which are devoid of high-GI (glycaemic index) carbohydrates. This concept is an extension of an earlier hypothesis put

forward by Schaefer (1971), who reported the emergence of acne in the Eskimos of North America following the adoption of a Western lifestyle. Schaefer proposed that the increase in acne prevalence in Eskimo groups was the result of the “shift to refined,.. rapidly absorbable carbohydrates”. This is in stark contrast to their earlier diet which was comprised primarily of meat and fish (Nestle, 1999). Although Russian settlers introduced the Eskimos to basic agriculture and carbohydrate foods (eg. barely, buckwheat, cabbage, and potatoes) some 70 to 100 years ago, these carbohydrates were generally low in GI and did not replace animal protein as the main source of energy (Smith, 2004). The emergence of acne to varying degrees in Eskimo groups appeared to coincide with the increase in the annual per capita consumption of refined sugar and flour, while the per capita consumption of protein derived from animal sources showed an inverse relationship (Schaefer, 1970; Schaefer, 1971). Since the relatively recent introduction of refined carbohydrates to the diet, the Eskimos have demonstrated faster growth (increased final height), earlier puberty and dramatic increases in the incidence of obesity, diabetes and heart disease (Schaefer, 1970; Schaefer, 1971; Bendiner, 1974).

A unifying feature of traditional diets is that they are naturally low in glycaemic load. As the GI can only be used to compare foods of equal carbohydrate content, the glycaemic load was later developed to characterise the glycaemic effect of whole meals or diets ($\text{GI} \times \text{available dietary carbohydrate}$). The glycaemic load may be interpreted as a measure of the blood glucose and insulin-raising potential, as it represents the rate of carbohydrate absorption and the quantity of carbohydrate consumed (Brand-Miller *et al.*, 2003). The glycaemic load may be modulated by altering the absolute amount of carbohydrate consumed or by selecting foods using the GI concept. Accumulating evidence suggests that the glycaemic load of Western diets has increased over recent years due to an; (1) increase in carbohydrate consumption as a consequence of dietary recommendations to decrease dietary fat and (2) as dietary trends appear to favour high GI foods (Slyper, 2004).

High glycaemic load diets have recently been implicated in acne aetiology due to their ability to increase insulin demand and other factors associated with insulin resistance (eg. hyperphagia, elevated NEFA, obesity) (Cordain *et al.*, 2002). Clinical and experimental evidence suggests that insulin may increase androgen production and effect, through its influence upon steroidogenic enzymes

(Kristiansen *et al.*, 1997), gonadotrophin releasing hormone secretion (Willis *et al.*, 1996) and sex hormone binding globulin (SHBG) production (Haffner, 1996; Goodman-Gruen and Barret-Connor, 1997; Plymate *et al.*, 1998). Furthermore, insulin has been shown to decrease a binding protein for insulin-like growth factor-I (IGF-I), which may facilitate the effect of IGF-I on cell proliferation (Powell *et al.*, 1991). Altogether, these events may influence one or more of the four underlying causes of acne: 1) increased proliferation of basal keratinocytes within the pilosebaceous duct, 2) abnormal desquamation of follicular corneocytes, 3) androgen-mediated increases in sebum production, and 4) colonization and inflammation of the comedo by *Propionibacterium acnes* (Cordain *et al.*, 2002).

Support for a role of insulin in acne development can also be found in the high prevalence of acne in women with polycystic ovary syndrome (PCOS), a condition associated with insulin resistance, hyperinsulinemia and hyperandrogenism (Franks, 2003). Insulin resistance is believed to be the underlying disturbance in PCOS, as it generally precedes and gives rise to the cluster of endocrine abnormalities that characterise PCOS (increased androgens, increased IGF-I and decreased SHBG) (Dunaif *et al.*, 1989). Treatments for PCOS now include oral hypoglycaemic agents which improve insulin sensitivity, restore fertility and alleviate acne (Ciotta *et al.*, 2001; Kazerooni and Dehghan-Kooshkghazi, 2003).

The extent to which acne is related to the typical Western diet is controversial, but the future holds promise of a shift in paradigm. A recent study by authors (Smith *et al.*, 2007), has found that a low glycaemic load diet, which mimics the diets of acne-free populations, significantly reduced acne lesion counts when compared with a conventional high glycaemic load Western diet. The lessening of acne severity may be explained by improvements in metabolic-endocrine parameters following dietary intervention. The experimental diet reduced insulin resistance and androgen bioavailability, and increased levels of SHBG and IGF-binding protein-1, when compared to controls. However, these changes may also relate to modest weight loss which concurred with the increase in protein and reduction in glycaemic load. Post hoc analysis revealed that the effect of the low glycaemic load diet on acne and certain endocrine variables (insulin sensitivity, SHBG and androgen bioavailability) was

lost after statistically adjusting for weight loss. Although this study could not determine an aspecific effect of a low glycaemic load diet from that of weight loss, these findings are consistent with earlier suggestions of the association between hyperinsulinemia and acne.

Although confirmation of the diet-acne hypothesis will require larger-scale experiments, the present study suggests that the institution of a non-Westernised diet, adapted for modern times, may have a therapeutic effect on acne. As a basic principal, this type of diet should contain minimally carbohydrate-based foods, including a wide variety of fresh fruits, vegetables, wholegrains, lean meats, fish and seafood.

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